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TLMs.

Recent Advances in the Chemistry of Cellulose and Starch

edited by

J. HONEYMAN, M.A., Ph.D.

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1

INTRODUCTION TO THE CHEMISTRY OF CARBOHYDRATES

Throughout this book, as far as possible, the systematic nomenclature recommended jointly by American[1] and British[2] authorities has been followed. The roman numerals in this paper refer to structural formulae which have been grouped together at the end for ease of reference.

STEREOCHEMISTRY, DIAGRAMMATIC REPRESENTATION, AND TERMINOLOGY

Emil Fischer established the fundamental chemistry of the carbohydrates when, towards the end of the last century, he made an outstanding study[3] of the configurational relationships[4] within the monosaccharide group. The conception of the asymmetric carbon atom, as described simultaneously by Le Bel[5] and van't Hoff,[6] was used to account for the existence of various isomeric monosaccharides which showed differences in optical activity. All monosaccharides have been related[4,7] to D-(+)-glyceraldehyde (I) and L-(—)-glyceraldehyde (II), two optically active forms termed stereoisomers which differ from one another in the disposition of the four different groups or atoms attached by covalencies to the central asymmetric carbon atom.[8,9] A covalent bond is normally represented diagrammatically by means of a straight line, and molecular models of the ball-and-peg type can be constructed from this type of representation to illustrate spatial or stereochemical arrangements. In this type of model, atomic sizes and interatomic distances are grossly exaggerated, consequently where these factors are of importance, more accurate models of the Stuart, Catalin, or Courtauld type must be used. It is preferable to examine molecular configurations by using these three-dimensional models although, in practice, the planar projection formulae (e.g. I and II), originally introduced[3,4] by Emil Fischer, are used for showing relationships between carbohydrates. By convention, the projection formulae are derived from the tetrahedral formulae (e.g. I*a*) by twisting the hydrogen atoms and hydroxyl groups above the carbon–carbon chain and flattening the molecule; only rotation of the projection formula in the plane of the paper is permissible. Projection formulae show the configurations, D- or L-, at individual carbon atoms only and, since they do not describe the conformations of the molecules, they are unsuitable for precise studies of stereochemistry. For this reason, increasing use is now being made of conformational representations (e.g. I*b* and II*b*) which illustrate the relative spatial positions of the atoms or groups in three dimensions; the carbon chain lies in the plane of the paper and the broken lines and the thick wedges indicate the valencies projecting behind and in front of this plane respectively.[10] Since there is freedom of rotation about the single bonds in these acyclic molecules, an infinite variety of conformations becomes possible. In practice, only one static formulation is selected to represent the dynamic molecule, usually the preferred conformation of lowest energy in which non-bonded interactions between atoms and groups are at a minimum.

An aldose belongs to the D-series when the hydroxyl group on the penultimate carbon atom, the reducing group being numbered first, is represented on the right of the carbon chain in the Fischer projection formula (Figure 1). In the L-series, the hydroxyl group is of opposite configuration and is on the left of the carbon chain. Since the aldohexoses contain 4 asymmetric carbon atoms there are 16 stereoisomers, of which 8, in the D-series (Figure 1), are mirror images of the other 8 in the L-series. The symbols D- and L- refer to configuration and *not* to the sign of observed optical rotation, the conventions (*dextro*), or (+), and (*laevo*), or (−), denoting the latter. Thus D-(+)-glucose (III) is stereochemically related to D-(−)-fructose (IV) as revealed[11] by the fact that on heating with aqueous phenylhydrazine they both yield the same phenylosazone (V).

PYRANOSE, FURANOSE, AND ACYCLIC FORMS

The monosaccharides do not behave as normal aldehydes or ketones because of intramolecular reaction of the carbonyl group with a hydroxyl group to give a cyclic hemiacetal.[12] In the case of D-glucose, and other hexoses and pentoses, ring closure can occur with the hydroxyl groups at either $C_{(4)}$ or $C_{(5)}$ to give furanose or pyranose structures, respectively, each of which possesses an additional asymmetric carbon atom at $C_{(1)}$. This extra asymmetric carbon atom causes the existence of α- and β-isomers of the pyranose (VI) and of the furanose forms (VII). The term ‘ anomer ’ was introduced[13] to describe the α- and β-isomers and $C_{(1)}$ is now referred to as the anomeric carbon atom. Haworth[14] illustrated pyranose and furanose forms by hexagonal and pentagonal formulae, which have been widely adopted for illustrating the stereochemistry of the cyclic molecules.

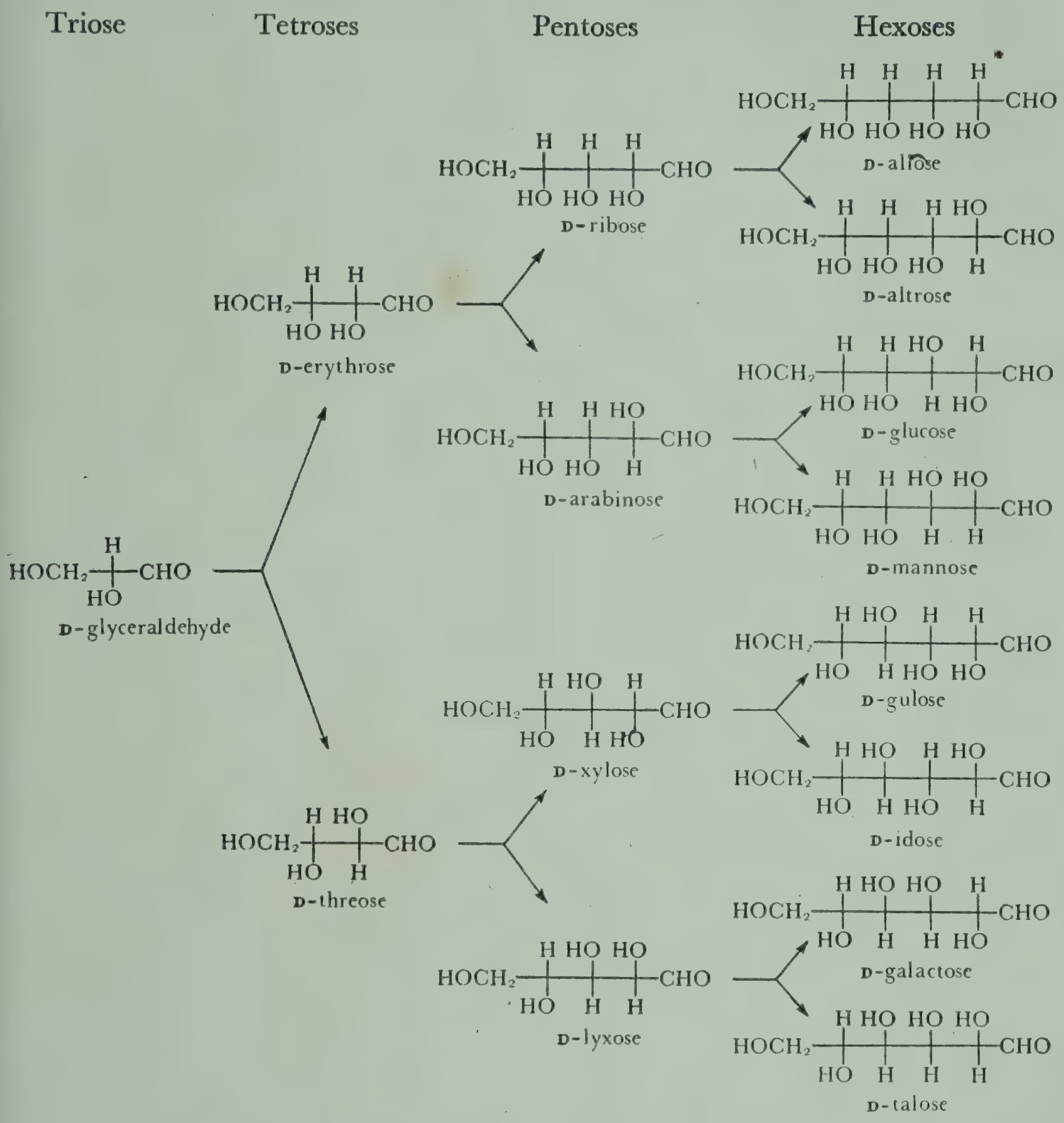

Triose
Tetroses
Pentoses
Hexoses
H H H H
HOCH₂ CHO
HO HO HO HO
D-allose
H H H
HOCH₂ CHO
HO HO HO
D-ribose
H H H HO
HOCH₂ CHO
HO HO HO H
D-altrose
H H
HOCH₂ CHO
HO HO
D-erythrose
H H HO H
HOCH₂ CHO
HO HO H HO
D-glucose
H H HO
HOCH₂ CHO
HO HO H
D-arabinose
H H HO HO
HOCH₂ CHO
HO HO H H
D-mannose
H
HOCH₂ CHO
HO
D-glyceraldehyde
H HO H H
HOCH₂ CHO
HO H HO HO
D-gulose
H HO H
HOCH₂ CHO
HO H HO
D-xylose
H HO H HO
HOCH₂ CHO
HO H HO H
D-idose
H HO
HOCH₂ CHO
HO H
D-threose
H HO HO H
HOCH₂ CHO
HO H H HO
D-galactose
H HO HO
HOCH₂ CHO
HO H H
D-lyxose
H HO HO HO
HOCH₂ CHO
HO H H H
D-talose

Figure 1. The D*-series of aldoses*

The α- and β-forms of D-glucopyranose (VI) were isolated[15] as distinct crystalline forms by crystallization from aqueous solution at room temperature and at the boiling point respectively. Whilst they have separate existence in the crystalline state, these isomeric substances, in common with other aldoses, equilibrate in solution to give a mixture consisting mainly of the α- and β-pyranose forms, along with smaller amounts of the furanose structures (VII) and the open-chain forms (VIII) which are probably hydrated as aldehydrols. The course of the equilibration process, termed mutarotation,[16] is accompanied by changes in the optical rotation of the solution as a result of the structural changes taking place. All monosaccharides are capable of existence in cyclic modifications but, in solution, the proportions of the various isomers vary according to the stereochemistry of the molecules and their environment, e.g. temperature, solvent, etc. Thus, in aqueous solution, D-ribose gives a high proportion of the *aldehydo* form[17] and D-fructose favours the furanose modification.[18]

It is not surprising, therefore, that the monosaccharides undergo chemical reactions to give products in which the pyranose, furanose, and open-chain forms are encountered. The nature of the product(s) will depend upon the reaction conditions, the nature of the reactant, and the stereochemistry of the monosaccharide. Thus, the conditions for the acetylation of D-glucose can be varied so as to yield either the α- or β-isomer of penta-*O*-acetyl-D-glucopyranose.[19–21] The acetylation reaction, using acetic anhydride and a catalyst, is often used for the assay of free hydroxyl groups. Acetates are also used for the isolation and identification of sugars because of the ease of preparation and the highly crystalline nature of the products. The catalysts used include zinc chloride, sodium acetate, pyridine, sulphuric acid, perchloric acid, and trifluoracetic anhydride. De-*O*-acetylation is a simple process involving treatment with either ammonia in methanol or catalytic amounts of sodium methoxide in methanol.

On treatment with ethanethiol in the presence of concentrated hydrochloric acid, aldoses generally give crystalline 1 : 1-diethyldithioacetals in high yields, e.g. D-glucose 1 : 1-diethyldithioacetal (IX).[22] The dithioacetals are useful for characterization purposes[23] and have been of considerable value as intermediates in the synthesis of various monosaccharide derivatives. For example, acetylation followed by removal of the thioalkyl groups by reaction with mercuric salts affords in aqueous solution the penta-*O*-acetyl *aldehydo* sugars,[24] but in the presence of alcohols, there are formed acetals, pyranosides, and furanosides.[25,26,27] By variation in the reaction conditions it is also possible to obtain thioglycosides. Raney nickel desulphurization affords initially 1-deoxy-1-thioalkylglycitols[28] and finally 1-deoxyglycitols.[29] A good method of descending the aldose series involves oxidation of the 1 : 1-diethyldithioacetals to the corresponding 1 : 1-diethylsulphonyl derivatives which, on treatment with base, disproportionate to give diethylsulphonylmethane and an aldose with one carbon atom less than the original.[30,31] Aldoses react stoichiometrically with cyanide under carefully controlled conditions to give open-chain cyanohydrins.[32] This reaction has been employed for the preparation of C^{14}-labelled sugars and also for determining minute amounts of reducing sugars, since the activity of the derived C^{14}-labelled cyanohydrin is directly proportional to the carbonyl groups originally present. This method has also been applied to the determination of molecular weights of oligosaccharides and polysaccharides.[32]

Many monosaccharides react in the furanose form with acetone containing a little acid catalyst to give *O-iso*propylidene derivatives as a result of condensation of the ketone with true *cis* hydroxyls on contiguous carbon atoms.[33] D-Glucose affords 1 : 2–5 : 6-di-*O-iso*-propylidene-D-glucofuranose (X),[34] a stable system of two virtually planar five-membered rings fused together at an angle of about 120°, in a V-shape, with no substituents directed into the V to cause steric pressure. Derivatives of *iso*propylidene are stable to alkali but extremely easily hydrolysed in dilute acid. Boric acid reacts similarly to acetone to give a complex at $C_{(1)}$ and $C_{(2)}$ of the glucofuranose structure.[35] Polyol–borate interaction is much more extensive in alkaline media and since the effect varies from one carbohydrate to another, extensive use is now being made of zone electrophoresis (ionophoresis) for the separation and identification of carbohydrates, including polysaccharides, from their characteristic rates of movement on paper and other media in the presence of various buffers, notably alkaline borate.[36,37] Certain mixtures of carbohydrates can be separated by preferential adsorption on the borate form of ion-exchange resin;[38] thus 3-*O*-methyl-D-glucose is strongly absorbed whereas 2-*O*-methyl-D-glucose is not.[39] A paper ionophoretic separation of a homologous series of reducing oligosaccharides as their *N*-benzyl-glycosylammonium ions has been described.[40] Less frequently, condensation of acetone with 1 : 3-*cis* diols to form a six-membered *m*-dioxane has been encountered (e.g. methyl 4 : 6-*O-iso*propylidene-α-D-glucopyranoside).[41] On the other hand, benzaldehyde usually reacts in this way, because the bulky phenyl group is better accommodated on a buckled six-membered ring than on a virtually planar five-membered ring, and thus with D-glucose the 4 : 6-*O*-benzylidene derivative (XI) is encountered.[42]

The separation, analysis, and identification of carbohydrates and their derivatives have been considerably improved by the use of paper chromatography[43,44,45] and column chromatography on cellulose,[46] Celite,[47] Celite–charcoal,[48] charcoal,[49] charcoal–borate,[50] charcoal–molybdate,[50] clays, and various other materials.[51]

When reducing sugars are dissolved in methanol containing a little acid catalyst, a mixture of isomeric cyclic hemi-acetals, termed methyl glycosides, is formed.[52] In contrast to the dimethyl acetals isolated from normal aldehydes or ketones, they contain only one methyl group. Reaction occurs at the anomeric carbon atom to give a mixture, in equilibrium, of methyl α- and β-pyranosides and methyl α- and β-furanosides, the proportions depending upon the reaction conditions and the stereochemistry of the reducing sugar. The glycosides can be separated by chromatography which therefore permits the analysis of a mixture of glycosides. Using this technique, the optimum conditions for the isolation of the four methyl D-galactosides have been determined (Table 1).[53] As in the case of D-glucose, furanosides (XIII) are formed initially and are then converted into the pyranosides (XII); the β-isomers appear before the α-isomers (XII, XIII). Conclusive evidence for the pyranoside and furanoside structures was first obtained by methylation of the free hydroxyl groups with either dimethyl sulphate and alkali,[54,55] or silver oxide and methyl iodide,[56] followed by oxidation and identification of the dibasic acids so formed.[57] Hirst[58] found *xylo*trimethoxyglutaric acid (tri-*O*-methylxylaric acid) in the nitric acid oxidation products of a methylated α-D-glucoside, thus proving that it was methyl 2 : 3 : 4 : 6-tetra-*O*-methyl-α-D-glucopyranoside, formed from methyl α-D-glucopyranoside (XII). The more convenient Malaprade periodate reaction[59] was later adapted by Hudson and his collaborators[60] for determining the ring structures of the glycosides.[61,62] On oxidation with sodium metaperiodate at room temperature and in the dark, hexopyranosides (e.g. XII) consume 2 moles of the oxidant by cleavage of the $C_{(2)}$—$C_{(3)}$ and $C_{(3)}$—$C_{(4)}$ bonds with the liberation of formic acid from $C_{(3)}$ and the formation of a dialdehyde (XIV) containing only two asymmetric carbon atoms. The characterization of the configurations at the anomeric carbon atom and at $C_{(5)}$ have been achieved by the isolation of crystalline derivatives from the dialdehydes (XIV) by oxidation to dicarboxylic acids (XV)[60] or by reduction to triols

Table 1: The optimum conditions for the formation of methyl D-galactosides

D-Galactoside	Time (hr)	HCl (per cent)	Temperature	Yield
α-Furanose	3	4	reflux	73
β-Furanose	6	0·5	room	33
α-Pyranose	20	4	reflux	40
β-Pyranose	6	0·5	room	50

(XVI).[63] Oxidation of hexofuranosides (e.g. XIII) with 2 moles of periodate gives 1 mole of formaldehyde, but no formic acid; the trialdehyde (XVII) formed contains a malondialdehyde group which in the presence of excess of periodate is further oxidized at the activated hydrogen atom attached to $C_{(4)}$*.[64,65]

The dialdehydes (XIV) resulting from the oxidation of the hexopyranosides exist in equilibrium with cyclic hemi-acetals (XVIII) as revealed by catalytic hydrogenation of only 1 aldehyde group in the presence of palladium and of 2 aldehyde groups when nickel was used.[66] Advantage was taken of the fact that maltose cannot form a furanoside to separate it, a 1 : 4-dihexosaccharide, from laminaribose, a 1 : 3-dihexoside, which rapidly forms a furanoside in methanolic hydrogen chloride. The products were subsequently separated on a charcoal–Celite column.[67]

CONFORMATIONAL ANALYSIS

Further discussion of the chemistry of carbohydrates requires a knowledge of the conformations or shapes of the molecules. In the case of an ethane derivative carrying large (L), medium (M), and small (S) groups on each asymmetric carbon atom, the projection formulae show that *erythro* (XIX) and *threo* (XX) configurations are possible. In the preferred conformations of lowest energy,[10,68] the large, medium, and small groups are staggered on either side of the zigzag carbon chain (XIX*a*; XX*a*) (see p. 2). Eclipsed groups (XXI) are clearly unfavourable since non-bonded interactions are greater than in the staggered conformations as shown by viewing the projections along the $C_{(1)}$-to-$C_{(2)}$ bonds (XIX*b*; XX*b*). *Erythro* isomers should be more stable than the *threo* types, a fact which is borne out experimentally, e.g. the racemization of substituted succinic acids gives more *erythro* than *threo* isomer.[10]

The preferred conformations erythritol (XXII*a*) and D-threitol (XXIII*a*) show that the hydroxyls at $C_{(2)}$ and $C_{(3)}$ are closer together in the *threo* isomer (XXII*b*) than in the *erythro* isomer (XXIII*b*), a point which is concealed by the projection formulae (XXII; XXIII) since the latter are only intended to show the D- or L-configurations at individual carbon atoms (see p. 2).

When D-glucose is reduced by sodium borohydride, a polyhydric alcohol termed sorbitol (D-glucitol) (XXIV) is obtained[69] and the reaction can be used quantitatively by determining the uptake of hydrogen.[70] The latter is obtained by acidifying both the reaction mixture and a blank determination, and estimating the difference in volumes of hydrogen liberated. Estimations of the rates of reduction of various D-glucose derivatives by potassium borohydride have revealed[71] that 3-*O*-substituents such as *O*-methyl and *O*-glycosyl sterically hinder the

$$KBH_4 + 4R.CHO \longrightarrow KB(OCH_2.R)_4 \xrightarrow{H_2O} 4HOCH_2.R$$
$$(KBH_4 + 3H_2O + HCl \longrightarrow H_3BO_3 + KCl + 4H_2, \text{ blank})$$

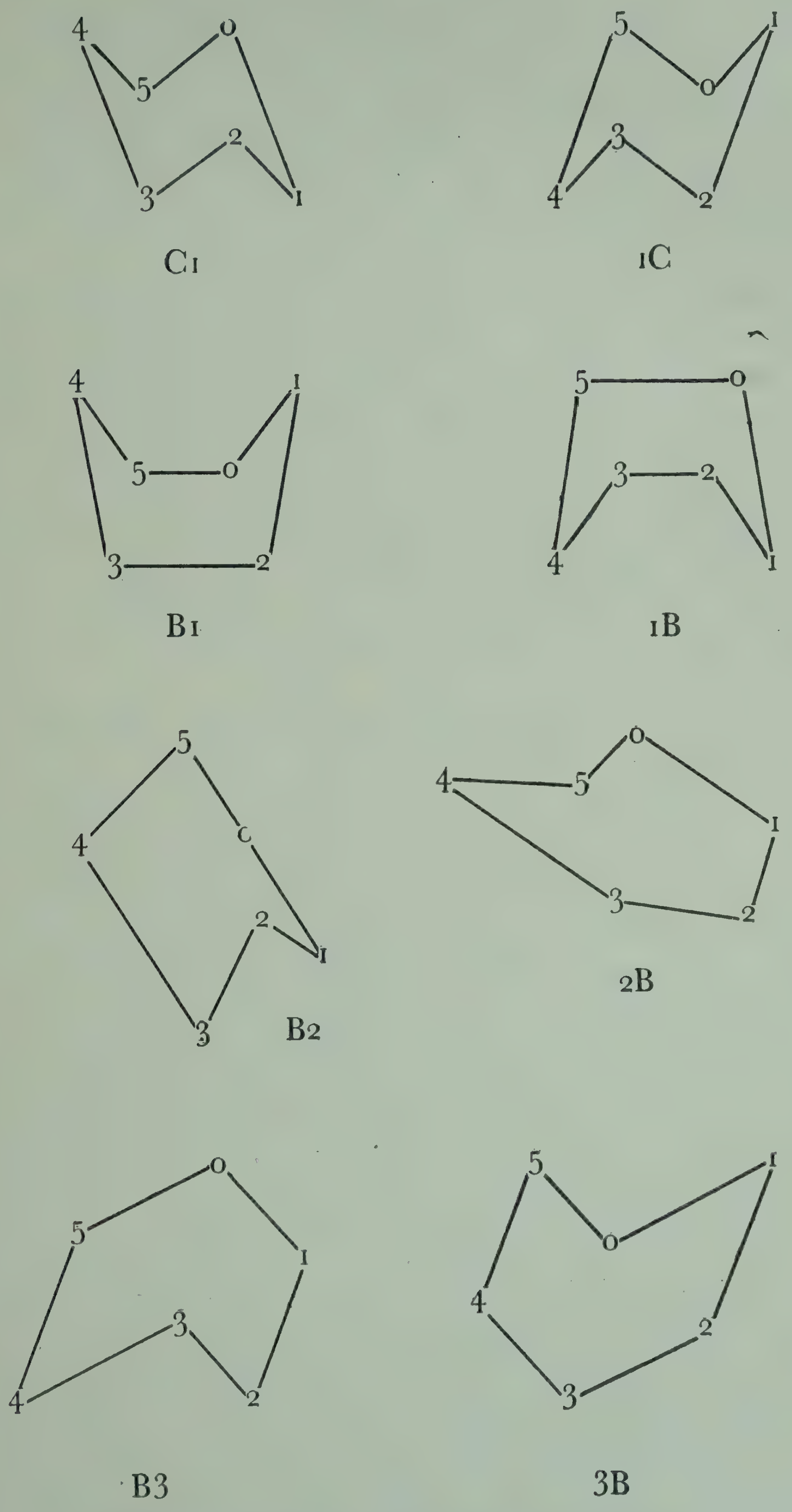
reaction. The *aldehydo* form of D-glucose in its staggered zigzag conformation (XXV) shows the close proximity of the $C_{(3)}$-*O*-substituents to the carbonyl group.

The projection formula of sorbitol (XXIV), like those of other polyols, does not show the preferred conformation of the molecule (XXVI) and makes the interpretation of certain reactions difficult. Thus, Schwarz[72] has shown that periodate attacks preferentially the *threo* hydroxyls of acyclic polyols. Where there is no steric hindrance *cis*-glycols react more rapidly than *trans*-glycols with periodate to form an intermediary complex (XXVII), involving a five-membered ring, which then breaks down with cleavage of the carbon–carbon bond to give two carbonyl groups and iodate.[73] Molecular models suggest that the ring of the complex is slightly buckled. The preferred conformations of the acyclic polyols (e.g. XXVI) reveal that *erythro*-hydroxyls are *trans*, whereas the *threo*-hydroxyls are virtually *cis* and hence the latter undergo preferential attack by periodate. This conclusion is of great importance in understanding the chemical reactivity of polyols and acyclic carbohydydrates, and is best comprehended by examining molecular models.

Lead tetraacetate reacts in a similar manner to periodate.[74] Acetone also reacts preferentially with *threo*-hydroxyls of acyclic polyols to give *iso*propylidene derivatives (e.g. 1 : 2–3 : 4–5 : 6-*O*-*iso*propylidene-D-sorbitol).

D-Glucofuranose as represented by the pentagonal formulation (VII) is essentially correct since the furanose ring is essentially planar. However, the pyranose form as depicted by the hexagonal formulation (VI) is a simplification of the actual shape of the molecule.[15] Like *cyclo*hexane derivatives, the ring is buckled and it can adopt either boat or chair conformations in order to avoid internal strain. Because of the presence of the ring oxygen atom, two chair-forms and six boat-forms are possible (Figure 2).[75] This type of isomerization and the factors governing which conformation is preferred are of fundamental importance in interpreting both chemical and physical properties of carbohydrates.

The preferred conformation will be that of lowest energy and it is governed by the non-bonded interactions between substituents on the pyranose ring.[75] The repulsive effects due to the hydroxyl


4
0
5
2
3
1
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0
3
2
4
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4
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1
3
2
0
5
1
3
4
2
5
1
0
4
2
3

C1 1C

B1 1B

B2 2B

B3 3B

Figure 2. The boat and chair forms of pyranose rings

groups are the main factors to consider here. Since strain is least in the chair forms (C1 and 1C) the boat conformations are not favoured except in very special circumstances. True *cis* groups (eclipsed) cannot occur in the chair conformations, but are found in the boat forms, and the instability of the latter can be attributed to non-bonded repulsion between these groups and between those at the flagpoles (Table 2).[76] During the course of a reaction, however, a molecule may be excited and take up a less favoured conformation but one which is necessary before reaction can occur.

Table 2: Distances between atoms attached to the pyranose ring

Position		Projected valency angle (degrees)	Interatomic distance (Å)	
			H—H	O—O
On adjacent carbon atoms	True *cis*	0	2·27	2·49
	Distorted *cis* or *trans*	+60; −60	2·49	2·83
	True *trans*	180	3·06	3·66
	Bow and stern flagpoles of a pyranose boat		1·83	1·61

The substituents on the pyranoside ring are of two types, equatorial substituents lying in the general plane of the ring and axial substituents which are orientated normal to this plane. Axial substituents are shown attached by valencies in solid lines and equatorial valencies by broken lines, in order to emphasize their differences in stereochemistry, reflected in their selective reactivity. In contrast to equatorial groups, axial groups introduce an element of instability because of increased repulsive effects as a consequence of their closer proximity to other axial substituents on the ring. Such considerations led Reeves[75] to the prediction that D-glucopyranose exists in the C1 conformation (XXVIII; OH's equatorial) rather than the 1C conformation (XXIX; OH's axial); the C1 form is transformed into the 1C form by moving $C_{(1)}$ up and $C_{(4)}$ down and, in the transition, axial groups become equatorial and vice versa. Support for the validity of this concept was obtained by Reeves[75] in an extensive investigation of the complexing of cuprammonium with various hexapyranosides. This reaction is based on the premise that cuprammonium hydroxide $(Cu(NH_3)_4(OH)_2)$ combines with *cis*-glycols to give a complex involving a five-membered ring containing copper. The relative positions of the two oxygen atoms of the glycol group were located by changes in the optical rotation and conductance of the solution. Complexing decreases with increasing distance between the hydroxyls; true *cis*-glycols are highly reactive (e.g. *erythro*-dihydroxyfuran; XXX) as shown by the increase in conductance, whereas no detectable reaction occurs with true *trans*-glycols (e.g. methyl 4 : 6-*O*-benzylidene α-D-altropyranoside; XXXI). Distorted *cis*-glycols are detected by a large rotational shift which has been ascribed to the formation of an asymmetric, five-membered ring which exerts a large rotational moment. The sign of the rotational shift depends upon the sign of the angle between the projected valencies of the hydroxyl groups, illustrated by the behaviour of 2-*O*-methyl (XXXII) and 4-*O*-methyl (XXXIII) derivatives of methyl β-D-glucopyranoside.[75] The experimental data (Table 3) agrees with

Table 3: The reaction of cuprammonium with various *O*-methyl β-D-glucopyranosides

Methyl β-D-glucopyranoside	Rotational shift (degrees)	Increase in specific resistance	Behaviour
4-*O*-Methyl	−2,020°	—	laevo
4 : 6-Di-*O*-methyl	+1,990°	52	laevo
2-*O*-Methyl	+2,190°	67	dextro
3-*O*-Methyl	− 83°	16	no complex
2 : 3-Di-*O*-methyl	− 9°	14	no complex
6-*O*-Methyl	+435°	70	*dl*-intermediate

the assignment of the C1 conformation to D-glucopyranosides, a form that has also been attributed to the glucose units of cellulose. The starch component, amylose, differs from cellulose in that it has limited solubility in cuprammonium, although it might be expected to react similarly; this and other data suggest that the D-glucose units in amylose are not in a chair conformation.[77]

Differences in the reactivity of hydroxyls in axial and equatorial positions are well illustrated by Aspinall and Zweifel's study of methyl 4 : 6-*O*-benzylidene α-D-mannoside (XXXIV) and 1 : 6-anhydro-β-D-mannopyranose (XXXV), which undergo selective esterification with 1 mole of toluene-*p*-sulphonyl chloride in pyridine at the less-hindered equatorial hydroxyls.[78] The primary alcohol group at the equatorial $C_{(6)}$ of D-glucose is the most reactive in esterification reactions; thus heating D-glucose in 50 per cent acetic acid yields 6-*O*-acetyl-D-glucose in good yield.[79]

The stereochemistry of D-glucopyranose is such that acetyl groups can, in the influence of alkali, migrate from one hydroxy group to another via an intermediary *ortho* ester (XXXVII) in the chair form of a strainless six-membered ring. Thus, 1 : 2 : 3 : 4-tetra-*O*-acetyl-β-D-glucose (XXXVI) is readily transformed into the 1 : 2 : 3 : 6-isomer (XXXVIII).[80,81] Methylations of methyl 2 : 3-di-*O*-acetyl-6-*O*-trityl-α-D-glucopyranoside (XXXIX) and methyl 2 : 3 : 4-tri-*O*-acetyl-α-D-glucopyranoside (XL) with silver oxide and methyl iodide, were accompanied by acetyl migration to give the corresponding 2-*O*-methyl derivatives.[81,82] Similar migrations of phosphate and nitrate groups have been observed.

The α- and β- isomers of methyl 4-*O*-methyl-D-glucopyranosides (XXXII) have been synthesized,[83,84] and they serve as model substances for investigating the reactions of cellulose and starch.

FURTHER REACTIONS OF MONOSACCHARIDES AND THEIR DERIVATIVES

The Reactions of Aldoses with Mineral Acids

Strong mineral acids dehydrate aldopentoses (XLI; $R = H$) to form furfural (XLII; $R = H$), and aldohexoses (XLI; $R = CH_2OH$) to form 2-hydroxyacetylfuran (XLIII), 5-hydroxymethylfurfural (XLII; $R = CH_2OH$), and laevulinic acid (XLIV):[85,86] colorimetric estimations of pentoses and hexoses are largely dependent on the formation of these products. Condensation of monosaccharides to give disaccharides and higher molecular weight materials can be catalysed by mineral acids.[87–96] In the case of D-glucose this process, generally referred to as reversion, gives rise to 1 : 6-glycosidic linkages predominantly, although 1 : 1-, 1 : 2-, 1 : 3-, 1 : 4-, and 1 : 5-linked diglucosaccharides have also been recognized (Table 4). Consequently, the partial acid hydrolysis of a polysaccharide for the identification of oligosaccharide fragments requires careful scrutiny in order to avoid anomalous results in this type of linkage analysis.

Table 4: The diglucosaccharides formed on reversion of D-glucose with acids

	References
6-*O*-α-D-glucopyranosyl-D-glucose (*iso*maltose)	92, 87
6-*O*-β-D- ,, ,, (gentiobiose	93, 94, 87
4-*O*-β-D- ,, ,, (cellobiose)	87
4-*O*-α-D- ,, ,, (maltose)	87, 94
2-*O*-β-D- ,, ,, (sophorose)	87
2-*O*-α-D- ,, ,, (kojibiose)	96
3-*O*-α-D- ,, ,, (nigerose)	87, 89, 95
5-*O*-β-D- ,, ,, ...	88
β-D-glucopyranosyl β-D-glucopyranoside (β, β-trehalose) ...	87
α-D- ,, α-D- ,, (α, α-trehalose) ...	88

In the presence of acid, D-altrose, D-allose, D-idose and D-gulose rapidly equilibrate with substantial amounts of their 1 : 6-anhydro derivatives[97-99] which are held in the 1C conformation with an equatorial hydroxyl group at $C_{(3)}$ (XLV).[75] On the other hand, the remaining aldohexoses, D-glucose,[95] D-mannose, D-galactose, and D-talose form little, if any, of the 1 : 6-anhydro derivatives since they would give rise to an axial hydroxyl at $C_{(3)}$ (XLVI) and the repulsive force between this group and the anhydro ring is to a large extent responsible.

The Reactions of Monosaccharides with Alkali

In the presence of alkali or the hydroxyl form of an ion-exchange resin, the monosaccharides are rapidly transformed into other products by isomerization and fragmentation processes. In dilute alkali at room temperature, D-glucose undergoes the Lobry de Bruyn and Alberda van Ekenstein transformation,[100] known also as epimerization, to give, via intermediary enediols, D-mannose and D-fructose. D-Psicose (D-allulose, D-*ribo*hexulose) was also detected in small amount and is believed to arise by inversion of configuration at $C_{(3)}$ of D-fructose via the 2 : 3-enediol.[101] DL-Sorbose and other L-sugars arise from the prolonged action of alkali on D-glucose,[102,103] probably as a result of disproportionation of D-fructose by reverse aldol reaction to give dihydroxyacetone and D-glyceraldehyde which rapidly equilibrates with its L-isomer; recombination of these trioses is known to give DL-fructose and DL-sorbose.[104,105] Lactic acid and saccharinic acids also result from the action of alkali on monosaccharides (see *The Alkaline Degradation of Cellulose and Chemically Modified Cellulose*, p. 106).

The Acid Hydrolysis of Glycosides

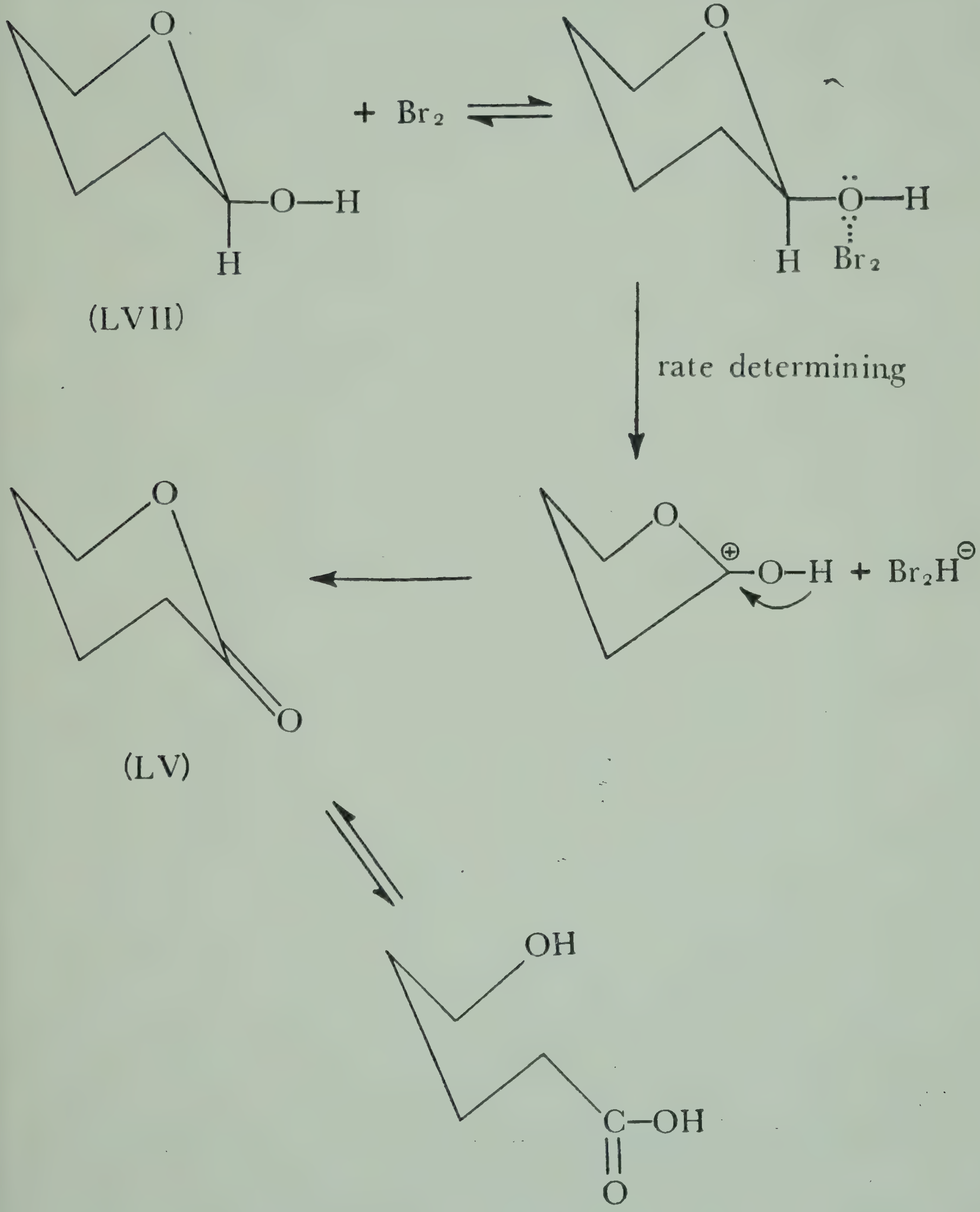
The mechanism of the acid-catalysed hydrolysis of pyranosides has been investigated by an isotopic tracer method using O^{18} and shown to proceed by unimolecular decomposition of their conjugate acids with fission of carbon–oxygen bonds only. All but two mechanisms were therefore excluded.[106] One process involves the formation of a conjugate acid (XLVII) by protonation of the glycosidic oxygen atom followed by dissociation of the O-to-$C_{(1)}$ bond to give a cyclic carbonium ion (XLVIII*a*) which would be stabilized by resonance with the oxonium ion (XLVIII*b*),[107] and thence by hydroxylation to the reducing sugar. In the alternative mechanism, protonation occurs at the ring oxygen atom, thus facilitating the dissociation of the ring O-to-$C_{(1)}$ bond to form an acyclic carbonium ion (XLIX). Conformational analysis has been used to explain the relative rates of hydrolysis of pyranosides (LIII) in terms of the first mechanism.[107-109] A methyl pyranoside with the methoxyl group at $C_{(1)}$ orientated in an equatorial position (e.g. methyl β-D-glucopyranoside) is hydrolysed more rapidly than its anomer with an axial methoxyl group (e.g. methyl α-D-glucopyranoside) which because of its hindered position would be more difficult to protonate. A rate-determining step is the formation of the oxonium ion (XLVIII*b*) which is believed to exist in the half-chair form (XLVIII*c*). Since its formation involves a small amount of rotation about the $C_{(2)}$—$C_{(3)}$ and $C_{(4)}$—$C_{(5)}$ bonds, the steric help or hindrance from substituents on the pyranose ring and the nature of the substituent at anomeric carbon atom and its configuration (axial or equatorial) will determine the rate. In accord with this theory, increasing size of an equatorial substituent at $C_{(5)}$ of methyl α-D-lyxopyranoside causes greater stability to acid hydrolysis: D-*glycero*-D-*manno*heptoside ($C_{(5)}$: CH(OH).CH_2OH) is greater than D-mannoside ($C_{(5)}$: CH_2OH) which is greater than D-lyxoside ($C_{(5)}$: H). The methyl pyranosides of 2- and 3-deoxypyranosides are more acid-labile than the normal, fully hydroxylated pyranosides and the 2 : 3-dideoxypyranosides show even greater lability. On the other hand, Shafizadeh and Thompson[110] prefer the second mechanism since the rate-determining formation of the carbonium ion will also depend on conformational factors and in support of their argument they cite the formation of acyclic substances on hydrolysis of certain glycosides. Thus, methyl 2 : 3 : 4-tri-*O*-acetyl-D-arabinopyranosides (L) with zinc chloride and acetic anhydride yield penta-*O*-acetyl-D-arabinose methyl hemiacetal (LI).[111]

Furanosides are hydrolysed at a rate much faster than pyranosides because non-bonded interactions are greater in a planar pentagonal furanoside (LII) than in a staggered pyranose chair form (LIII). However, δ-lactones (LV) are more labile than γ-lactones (LIV) because an *exo*-double bond stabilizes a five-membered ring whereas it reduces the stability of a six-membered ring because of the trigonal nature of the carbonyl carbon.[112]

Oxidations of Aldoses and Glycosides

Aldopyranoses are oxidized with buffered bromine solutions (pH 5·4 : 0°) with the abstraction of two hydrogen atoms from the anomeric carbon atom, to give initially the δ-lactone (LV) which then hydrolyses to the aldonic acid (LVI).[113] The mechanism may well involve an initial rapid and reversible formation of a halogen complex followed by a rate-determining transfer of a hydride ion

(Figure 3).[114] β-D-Glucose is more rapidly oxidized than α-D-glucose by a variety of reagents, such as potassium permanganate, sodium hypo-iodite and bromine water,[113,114] in agreement with the general observation that an axial hydroxyl group is less accessible to attack than the equatorial hydroxyl group. The use of chlorine for the bleaching and removal of non-cellulosic material from wood pulp involves losses of cellulose; its action on simple glycosides has



(LVII)

(LV)

(LVI)

Figure 3. The halogen oxidation of aldopyranoses

therefore been examined. Lindberg and his collaborators[115] found that methyl β-D-glucopyranoside (LVIII) reacted in a saturated aqueous solution of chlorine (about pH 1) with cleavage of the glycosidic bond. The principal product was D-gluconic acid (LIX) which was slowly oxidized to 5-ketogluconic acid. Acid hydrolysis was negligible under the experimental conditions employed. The same products were isolated from the action of aqueous chlorine on methyl α-D-glucopyranoside (LX), but the methoxyl group is axial and consequently the rate of reaction was much slower. The reaction mechanism is obscure, but it has been suggested that substitution of the anomeric hydrogen atom occurs with the formation of a chloro derivative which rapidly hydrolyses to give D-gluconic acid. On the other hand, Henderson[116] observed that when chlorine was allowed to react with methyl β-D-glucopyranoside (LVIII) in aqueous solution, buffered to pH 4·5, the major products were D-glucose, D-arabinose, carbon dioxide and oxalic acid. This process appears to involve at least two reactions, one of which gives rise to D-arabinose by oxidative cleavage of the $C_{(1)}$—$C_{(2)}$ bond of D-glucopyranoside to give a carbonate ester of D-arabinose (LXI), and the other giving D-glucose by oxidative cleavage of the glycosidic group. Acid hydrolysis of the glucoside was negligible under the reaction conditions employed. Cleavage of the pyranose ring of methyl 4-*O*-methyl-β-D-glucopyranoside (XXXII) by chlorine in aqueous solution at pH 9·5 has been noted.[84]

Bentley[114] has drawn a correlation between the conformation of the pyranosides, the configuration at $C_{(1)}$, the optical rotation, and the relative reactivity of anomeric glycosides to halogen oxidation. Thus for any D- or L-pyranose of stable C1 conformation, the more dextro-rotatory member of the anomeric pair will have its anomeric substituent in the axial position. Similarly for any pyranose of stable 1C conformation the more laevo-rotatory member of the anomeric pair will have the anomeric substituent in the axial position. The anomer with the glycosidic constituent in the axial position will in general be the least reactive towards halogen oxidation. Methyl α-D-arabinopyranoside (LXII; preferred conformation 1C) was oxidized by chlorine more rapidly than the β-anomer.[114]

Lindberg and Theander[117] oxidized methyl β-D-glucopyranoside (LVIII) with dichromate in the presence of oxalic acid because under these conditions cellulose yields an oxycellulose containing practically no carboxyl groups. Amongst the products methyl 3-*keto*-β-D-glucopyranoside (LXIV) and methyl 6-*aldehydo*-β-D-glucopyranoside (LXIII) were recognized. The conversion of glycosides into uronides (LXV) under the influence of alkaline solutions of bromine and iodine has been suggested.[118]

The Reactions of Glycosides with Alkali

In aqueous alkali, certain glycopyranosides undergo relatively large reversible changes in optical rotation as a result of changes in the conformation of the molecules because axially oriented hydroxyl groups tend to move into equatorial positions to reduce non-bonded interactions.[119] The stability of glycosidic linkages to alkali under strong conditions (10 per cent sodium hydroxide; 170° for several hours) in the absence of oxygen has been studied[120] because of its importance in relation to losses of cellulose during pulping under alkaline conditions. Preliminary results[120] show that degradation does occur and suggest that a mechanism similar to that undergone, under much milder conditions, by phenyl β-D-glucopyranoside (LXVI; R = CH_2OH) in which a phenoxide ion is eliminated by cleavage of the $C_{(1)}$—O bond by participation of the $C_{(2)}$-hydroxyl to give a 1 : 2-anhydro derivative (LXVII; R = CH_2OH). The latter is very unstable and reacts with water to give D-glucose (LXVIII; R′ = H; R = CH_2OH), with alcohols to form β-D-glucopyranosides (LXVIII; R′ = Me; R = CH_2OH) or internally with the $C_{(6)}$-hydroxyl to form 1 : 6-anhydro-D-glucopyranose (LXIX) which is usually the major product.[121,122] Lindberg[120] isolated sorbitol from cellobi-itol and lactitol, and 1 : 6-anhydro-β-D-galactose in low yield from lactitol, after treatment with alkali under severe conditions. McKinney[123] has suggested that a part of the sugar residues originally linked to lignin by phenyl glycosidic linkages are transferred to the cellulose structure during alkali treatment. Support for this hypothesis was obtained[124] by treating phenyl β-D-xylopyranoside (LXVI; R = H) with aqueous alkali in the presence of cellulose, when xylose units were subsequently attached to this polysaccharide, probably by reaction with the intermediary 1 : 2-anhydro-D-xylopyranose (LXVII; R = H).

The selective reactivity of the hydroxyl groups in methyl α-D-glucopyranoside (LX) was investigated by Wolfrom and his collaborators.[125] The mono-sodio derivative of this glycoside, obtained by treatment with sodium hydroxide (1·25 mole) in butanol, gave the 2-*O*-methyl ether on methylation with methyl iodide.

Xanthation and Related Processes

After treatment of the mono-sodio derivative of methyl α-D-glucopyranoside with carbon disulphide and then with methyl iodide, the product was recognized as the 2-*O*-(*S*-methyl dithiocarbonate),[125] also known as the xanthate (Figure 4). The selective reactivity of the $C_{(2)}$-hydroxyl in xanthation was thus revealed, and starch[126] and cellulose[127] are reported to behave similarly. Methyl 3 : 4-*iso*propylidene-β-D-arabinoside (LXX) was converted into the 2-*O*-(*S*-methyl dithiocarbonate) derivative which on distillation yielded, not the expected olefin (Chugaev-type elimination),

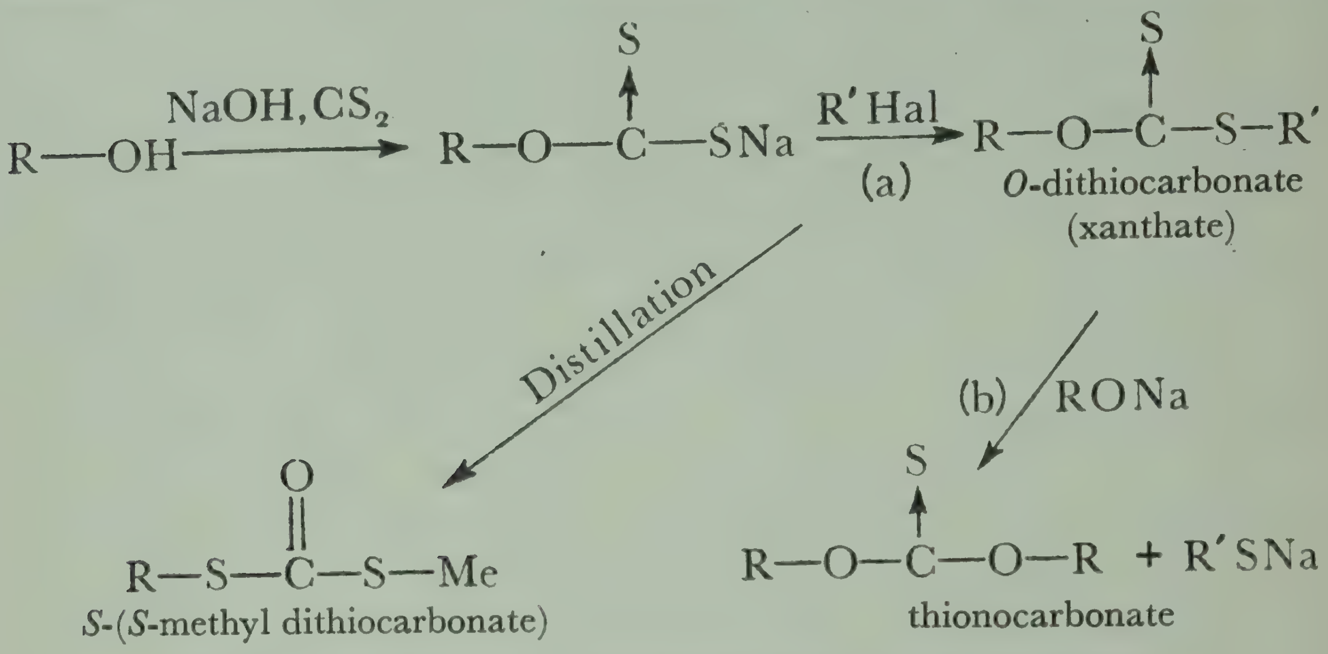


Figure 4. The formation of dithiocarbonates and derived products

but the isomeric 2-*S*-(*S*-methyl dithiocarbonate),[128] probably as a result of interference with the stereochemistry of the transition state. *S*-Methyl and *S*-trityl xanthates were obtained from reactions of the sodium xanthates with the corresponding halide derivatives, but ethyl, propyl, and butyl derivatives were not isolated because of their slow formation (reaction *a*) thus permitting reaction of the xanthates with unreacted sodium xanthate (reaction *b*) to form thionocarbonates which contain two monosaccharide units (Figure 4).[128]

Carboxymethyl and Hydroxyethyl Ethers

Carboxymethyl ethers of D-glucose have been prepared[129] by treating suitably protected glucose derivatives with sodium and the methyl ester of α-bromoacetic acid, followed by removal of the protecting groups. These synthetic products have been related to the products obtained by hydrolysis of *O*-carboxymethyl celluloses. Crystalline 2-*O*-, 3-*O*-, and 6-*O*-hydroxyethyl-D-glucose were obtained by reduction of these carboxymethyl ethers with lithium aluminium hydride[129] and also by column chromatography of a hydrolysate of hydroxyethyl cellulose.[130] Paper chromatography can be used for their identification and quantitative estimation.[129,130]

The 3-*O*-substituted derivatives were synthesized from 1 : 2–5 : 6-di-*O*-*iso*propylidene-α-D-glucofuranose (X). Reaction of the latter with ethylene oxide in the presence of aqueous alkali generally gives polyethylene oxide derivatives by further reaction of the introduced hydroxyethyl groups, although under carefully controlled conditions a good yield of the 3-*O*-hydroxyethyl derivative (LXXI) can be obtained.[131] The tosylation–iodination method[131] for determining primary alcohol units is not valid for hydroxyethyl derivatives of D-glucose and cellulose since these substituents are partially eliminated during the tosylation process.[131]

Nitrate Esters

The hydroxyl groups of glycosides can be converted into nitrate esters by using fuming nitric acid in chloroform, dinitrogen pentoxide in chloroform, or fuming nitric acid in acetic anhydride.[132–135] Reductive methods are generally used for the liberation of the hydroxyl groups from these nitrate esters; thus reaction with hydrazine gives good results.[136] Nitrate esters do not undergo simple saponification on treatment with alkali, but undergo nitrogen–oxygen and carbon–oxygen fission, often with extensive decomposition.[135,137,138] For example, alkaline hydrolysis of methyl 4 : 6-*O*-benzylidene α-D-glucopyranoside 2 : 3-dinitrate (LXXII) gave, under varying conditions, the 3-nitrate (LXXIII), methyl 4 : 6-*O*-benzylidene-α-D-glucopyranoside (LXXIV), methyl 4 : 6-*O*-benzylidene-2 : 3-anhydro-D-allopyranoside (LXXV) and an α-diketone (LXXVI). The 2-nitrate group in various D-glucopyranoside 2 : 3-dinitrate derivatives has been removed preferentially affording 3-nitrates.[138] A cellulose 3 : 6-dinitrate has been prepared by the action of pyridine–hydroxylamine on the 2 : 3 : 6-trinitrate.[139] Reaction of the nitrate group at $C_{(6)}$ of hexopyranosides usually involves carbon–oxygen fission with the elimination of nitrate. On treatment with sodium iodide in acetone, 6-deoxy-6-iodo derivatives are obtained from the 6-nitrates (e.g. LXXVII), whereas with the same reagent, nitrate groups on secondary carbon atoms are in some instances replaced by hydroxyl. Reaction of methyl 2 : 3 : 4-tri-*O*-acetyl-α-D-glucopyranoside 6-nitrate (LXXVII) with alkali gives methyl 3 : 6-anhydro-α-D-glucopyranoside (LXXVIII) in high yield[140] because of the participation of the $C_{(3)}$-hydroxyl in the elimination of the 6-nitrate group.

The Formazan Reaction

The formazan reaction was developed by Zemplén, Mester, and their colleagues, and has proved of value in the carbohydrate field for determining the presence or absence of ring structures in various hydrazones and osazones.[141–144] The reaction consists of the coupling of a diazonium compound in pyridine or alkaline–ethanol solution, with an acyclic aldose phenylhydrazone derived from a primary hydrazine. For the reaction to proceed, the presence of both the true Schiff base system (—CH = N—) and an imino hydrogen atom (= N—NH—) are essential (Figure 5). The reaction involves the formation of an intermediary diazohydrazone which then rearranges to the formazan. Acyclic ketose hydrazones (LXXIX), acyclic aldose hydrazones derived from asymmetric secondary hydrazines (LXXX) and all derivatives in which the hydrazone function is involved in ring formation (LXXXI) do not undergo the formazan reaction. The reversible oxidation of the coloured formazans into colourless tetrazolium derivatives[145] (Figure 5) is of considerable interest as an indicator in biological systems. Reaction of D-glucose with phenylhydrazine affords three isomeric crystalline phenylhydrazone derivatives, of which only one, the acyclic form (LXXXII) undergoes the formazan reaction,[141] the other two forms existing in cyclic modifications (e.g. LXXXI). The formazan reaction has also been applied to the phenylhydrazone derivatives of oxidized polysaccharides. Thus the phenylhydrazone of periodate

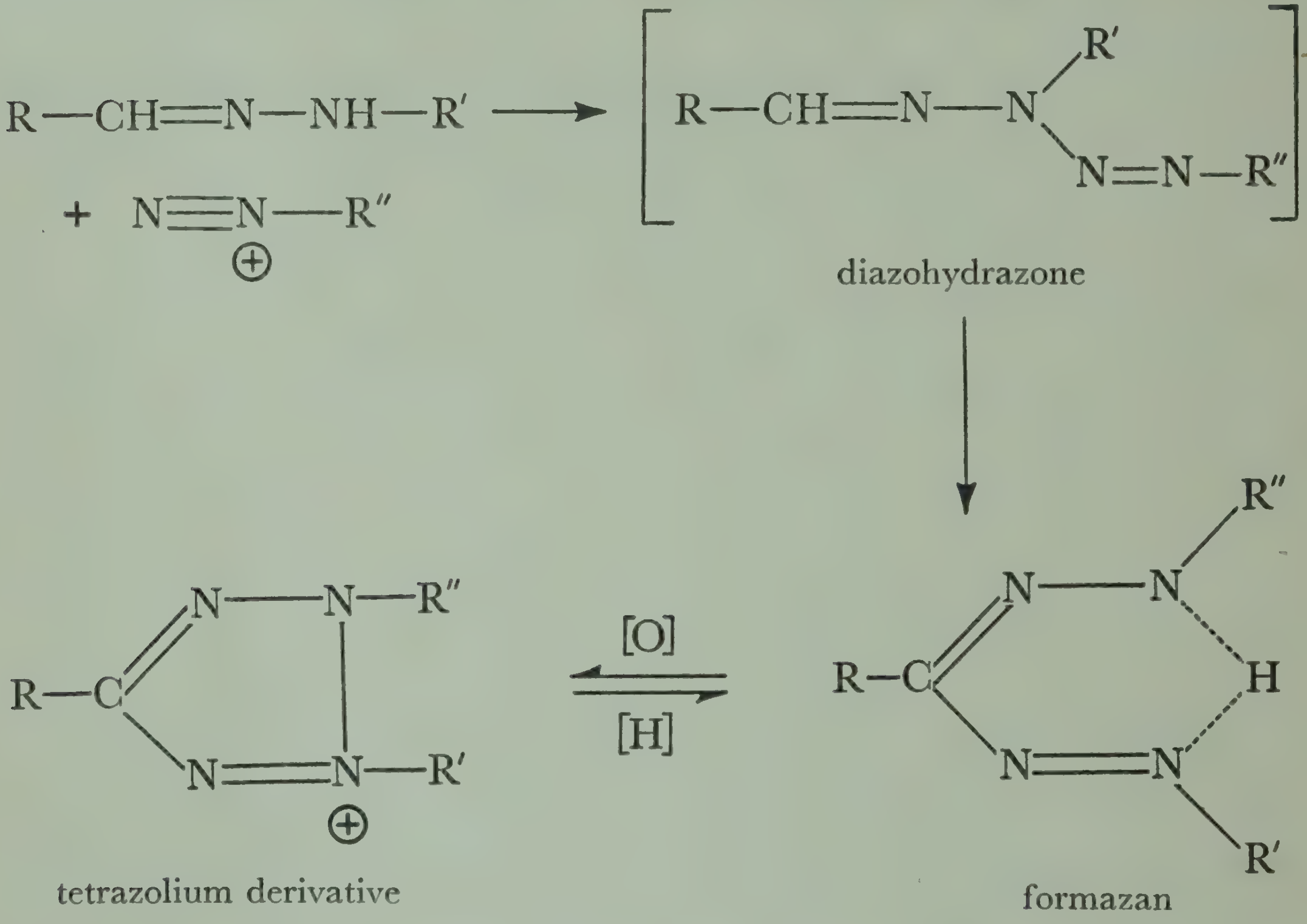


Figure 5. The formazan reaction

oxidized starch gives a formazan derivative, indicating the open-chain structure for the phenylhydrazone moiety.[143] Similar results were obtained with oxidized cellulose. Of the three possible structures suggested[146–148] for D-glucose phenylosazone (LXXXVIII, LXXXIX, XC), the acyclic structure (LXXXIX) has been shown to be the correct one,[148] as confirmed by a positive formazan reaction due to the presence of the —CH = N—NH—R structure at $C_{(1)}$.[143]

Glycosylamines

Aldohexoses, in common with other monosaccharides, react with amines to give glycosylamine derivatives which in the crystalline forms have separate existence in cyclic (LXXXIII) or acyclic modifications (LXXXIV), but which probably exist in solution in equilibrium one with another.[149] The glycosylamines undergo the Amadori rearrangement[150–153] under the influence of hydrogen ions to give 1-amino-1-deoxy-ketose derivatives, known as '*iso*glycosylamines', which can also occur in acyclic (LXXXV) or cyclic modifications (LXXXVI). These derivatives are readily transferred by loss of water into furfural derivatives (LXXXVII) and thence to brown products. The non-enzyme browning of foodstuffs is generally due to the interaction of sugars with amino acids by a similar series of reactions to those outlined above.[154–157]

Periodate Oxidation

A semi-micro procedure for determining the positions of substituted groups in reducing monosaccharides (e.g. methyl ethers and disaccharides) has been devised by studying the periodate oxidation of phenylosazone derivatives.[158] D-Glucose phenylosazone (LXXXIX) yielded mesoxaldehyde 1 : 2-bisphenylhydrazone (XCI), and formaldehyde. However, substitution of any one of the hydroxyl groups results in the absence of one of these products, thus indicating the position of substitution.

D-Glucose reacts mainly in the pyranose form with sodium metaperiodate with the rapid consumption of 3 moles of oxidant with the formation of 2 moles of formic acid and 2-*O*-formyl-D-glyceraldehyde (XCII).[159–161] Subsequent oxidation is dependent upon the rate of hydrolysis of this formyl ester which, when complete, will have resulted in the reaction of an extra 2 moles of periodate to give 1 mole of formaldehyde and a further 3 moles of formic acid (Figure 6). Other aldohexoses and aldopentoses are also oxidized in the pyranose form.[161] 1 : 4-Dihexosaccharides such as maltose (XCIII) and cellobiose are also oxidized in their pyranose forms by sodium metaperiodate to give, after complete reaction, 2 moles of formaldehyde, 1 mole of carbon dioxide, and 9 moles of formic acid, with the consumption of 11 moles of periodate.[162,163] This process proceeds through the oxidation of an intermediary malondialdehyde derivative (XCIV) and is believed to follow the route

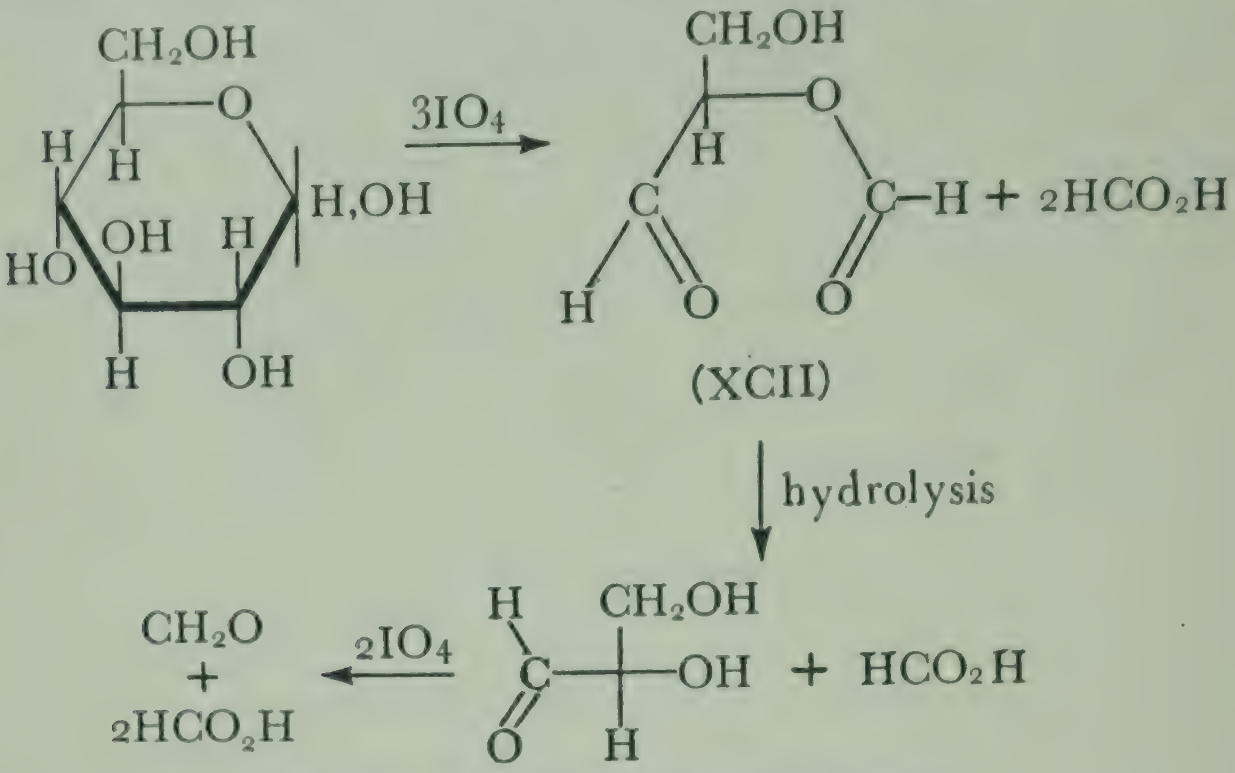


Figure 6. The periodate oxidation of D-*glucose*

indicated (Figure 7). For a further discussion of this ' over-oxidation ' process, see *The Hydrolytic and Oxidative Degradation of Cellulose*, p. 75, et seq. 1 : 2-Dihexosaccharides and 1 : 3-dihexosaccharides behave similarly and consequently quantitative micro-estimation of the carbon dioxide and formaldehyde produced under carefully controlled conditions can be used for the detection of 1 : 2-, 1 : 3-, and 1 : 4- linkages.[164,166] 1 : 6-Dihexosaccharides (e.g. melibiose) can be distinguished since they do not give rise to formaldehyde and carbon dioxide because malondialdehyde derivatives are not produced during the oxidation. The 1 : 2-linked dihexosaccharides (e.g. sophorose) can also be recognized since reduction with sodium borohydride prior to periodate oxidation prevents the formation of a malondialdehyde system and consequently carbon dioxide is not formed.[165]

The periodate oxidation of disaccharides and higher saccharides can be so controlled as to stabilize the formyl ester (XCV) formed from the reducing end-group and consequently the yield of formic acid produced during this reaction affords useful evidence for the structural investigation of carbohydrates.[167-170] This method has proved particularly effective in determining the average chain length per non-reducing end-group of the starch polysaccharides.[170]

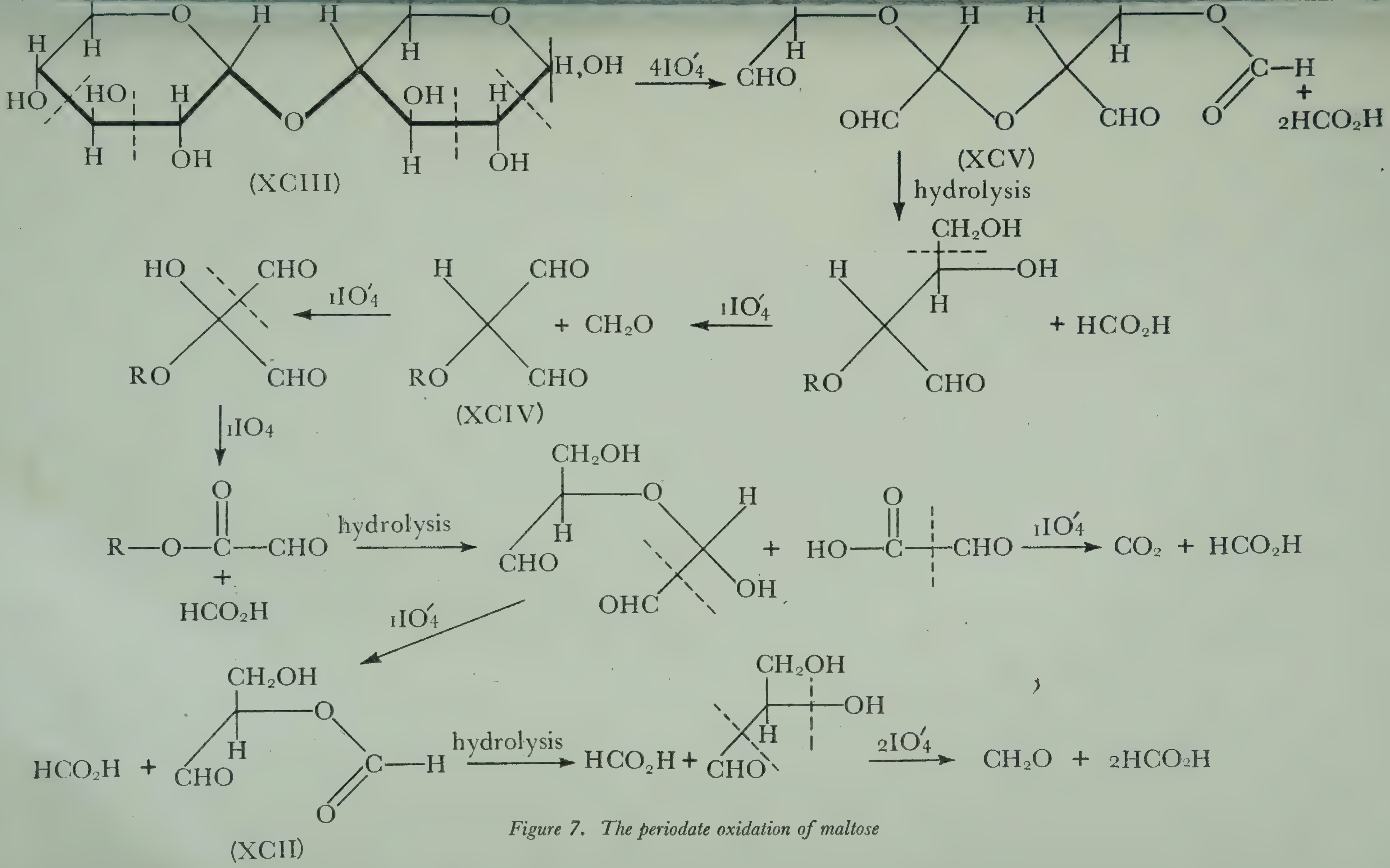
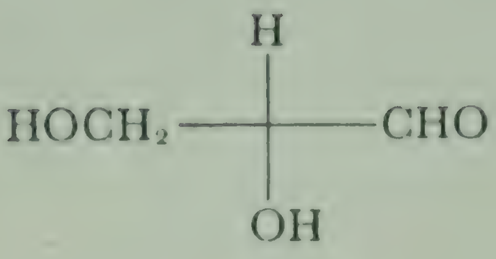
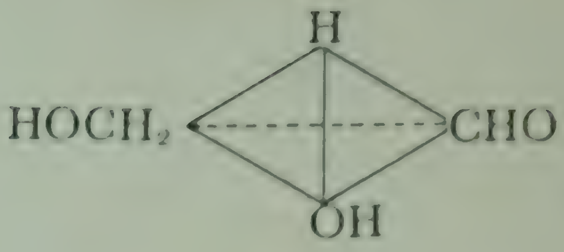

(XCIII)
4IO′4
(XCV)
+ 2HCO₂H
hydrolysis
+ HCO₂H
1IO′4
+ CH₂O
(XCIV)
1IO′4
1IO4
+ HCO₂H
hydrolysis
1IO′4
CO₂ + HCO₂H
1IO′4
(XCII)
hydrolysis
2IO′4
CH₂O + 2HCO₂H

Figure 7. The periodate oxidation of maltose



(I)



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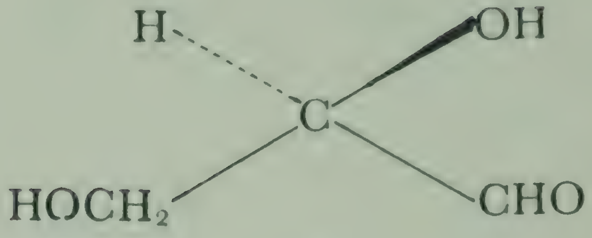

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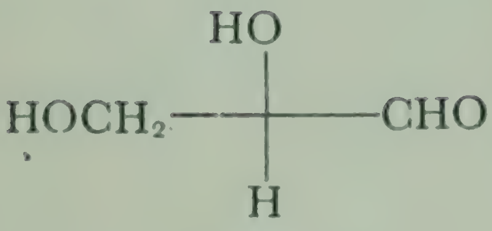

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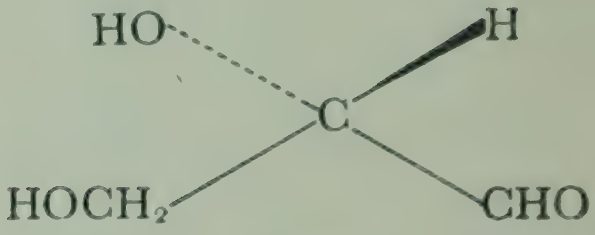

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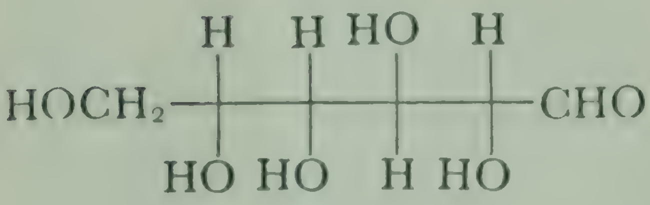
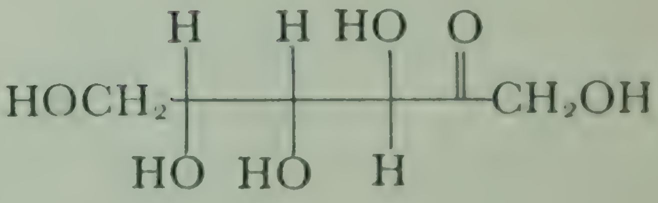
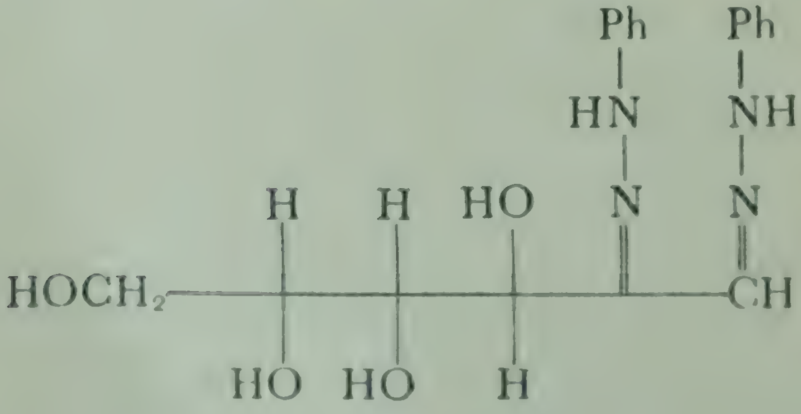


(III)



(IV)



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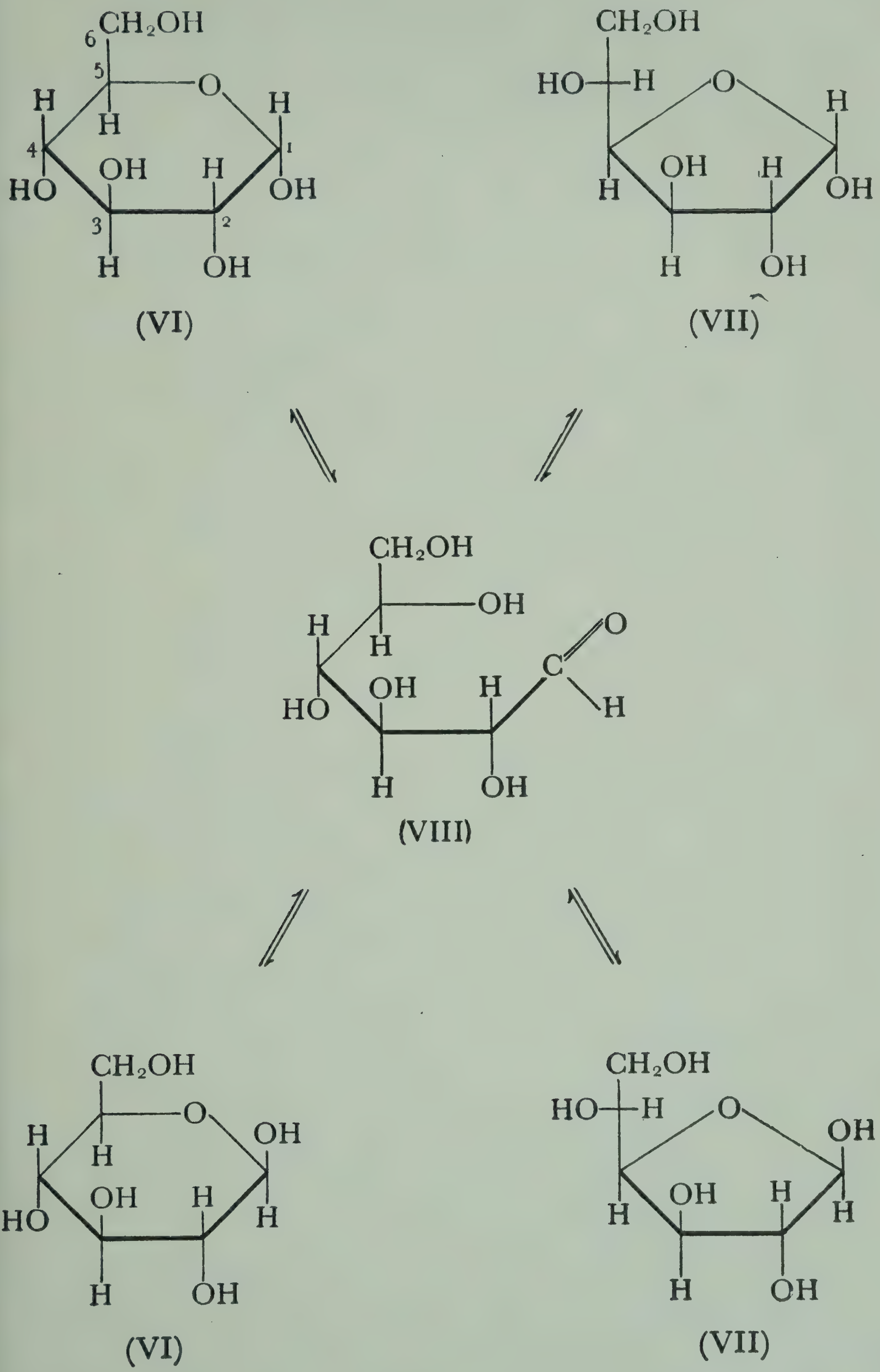
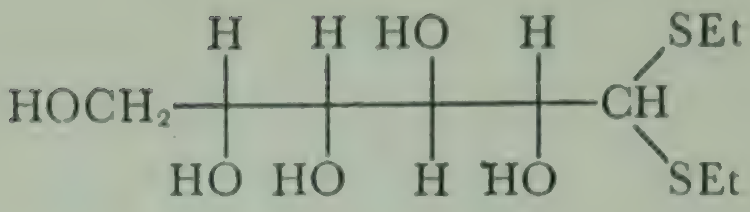


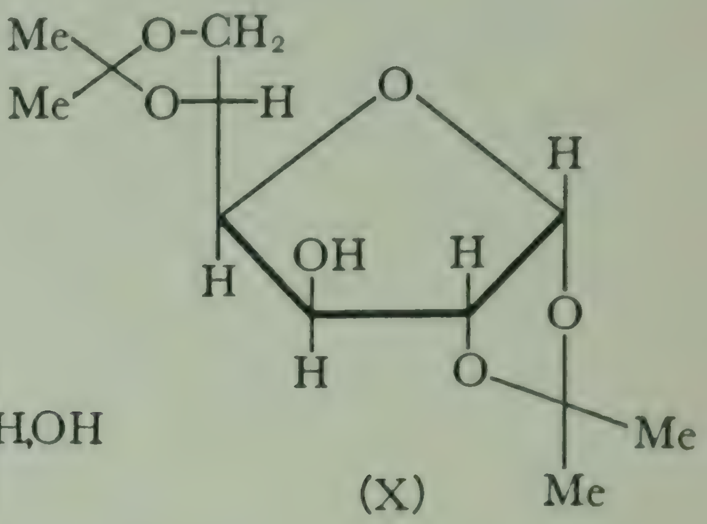
(VI) (VII)

(VIII)

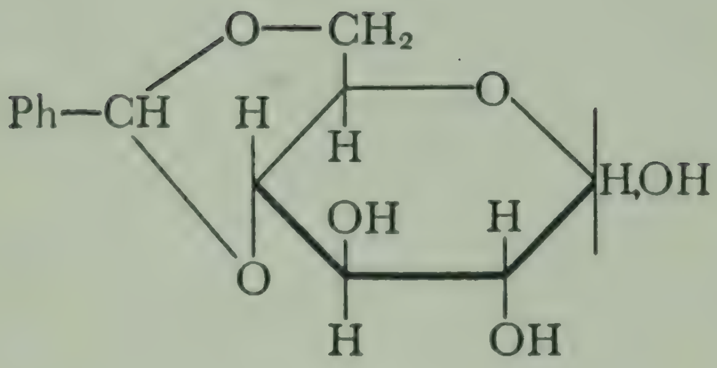
(VI) (VII)



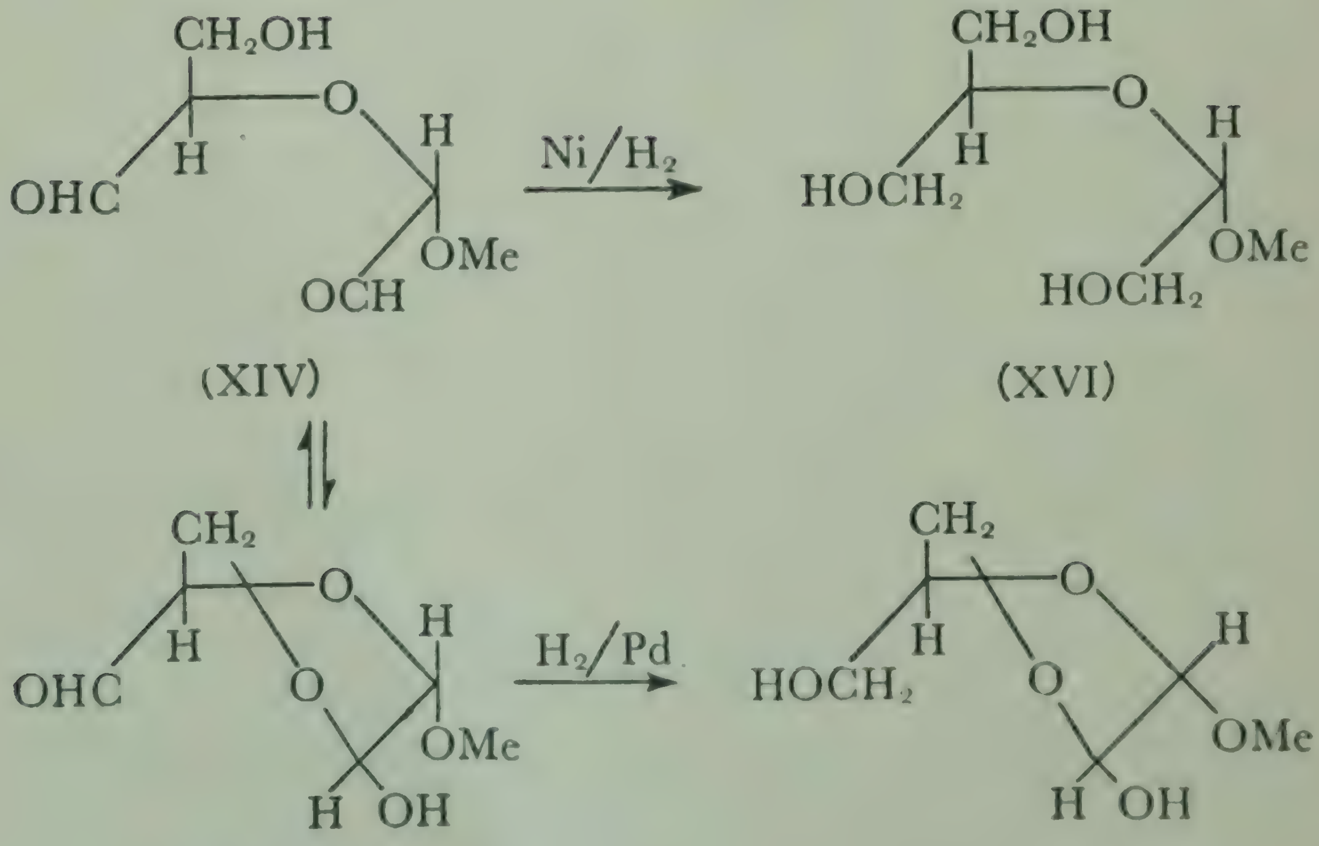
(IX)



(X)



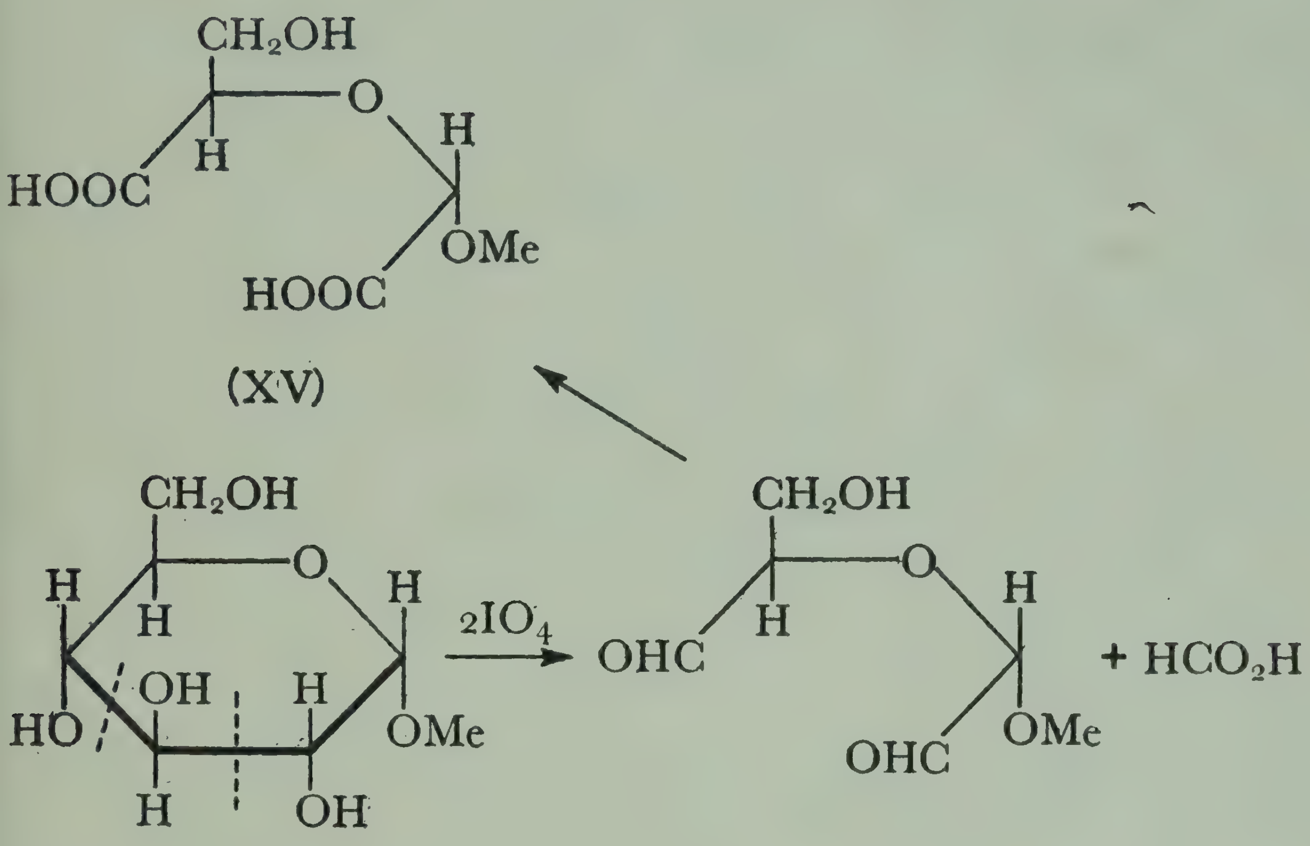
(XI)



(XIV)

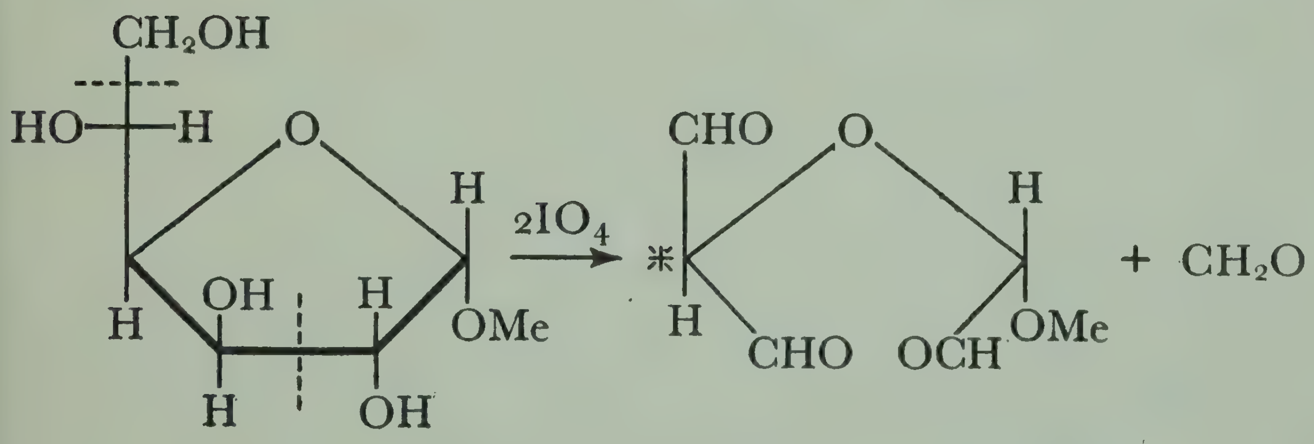
(XVI)

(XVIII)

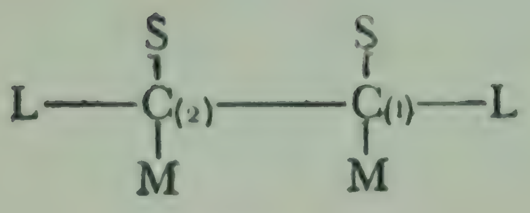


(XV)

(XII) (XIV)

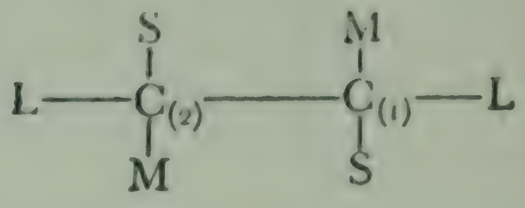


(XIII) (XVII)


S
S
L
C(2)
C(1)
L
M
M

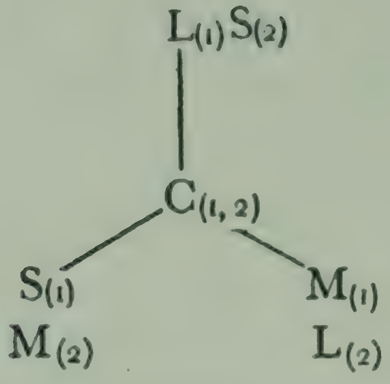
(XIX)

erythro

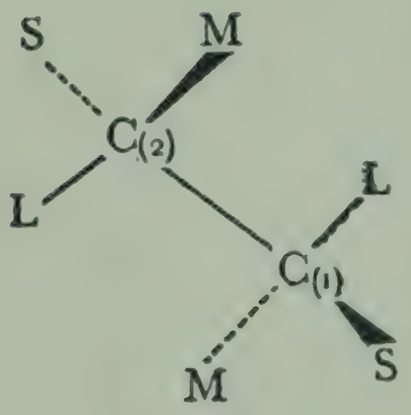

S
M
L
C(2)
C(1)
L
M
S

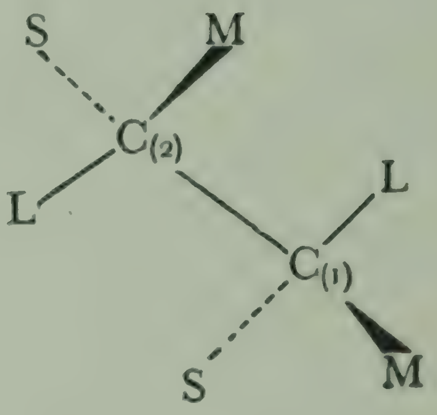
(XX)

threo


L(1) S(2)
C(1, 2)
S(1)
M(2)
M(1)
L(2)

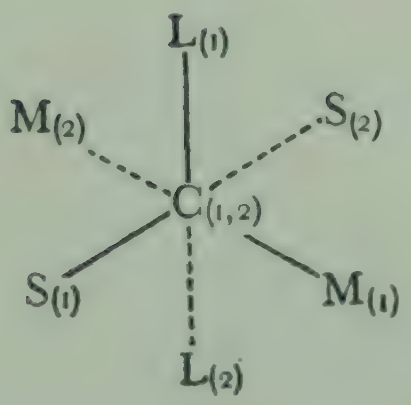
(XXI)

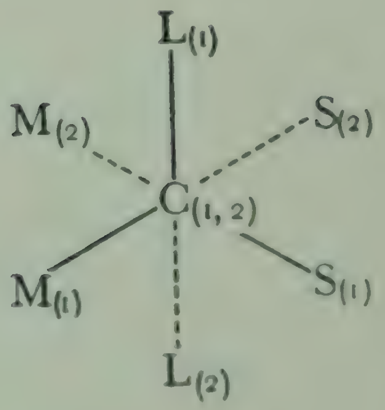

S
M
C(2)
L
L
C(1)
M
S


S
M
C(2)
L
L
C(1)
S
M

(XIXa)

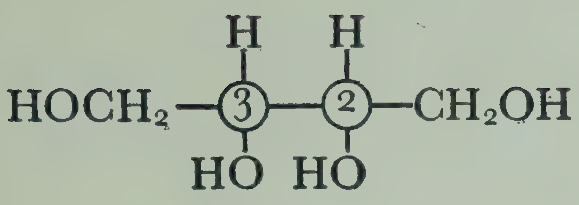
(XXa)


L(1)
M(2)
S(2)
C(1, 2)
S(1)
M(1)
L(2)


L(1)
M(2)
S(2)
C(1, 2)
M(1)
S(1)
L(2)

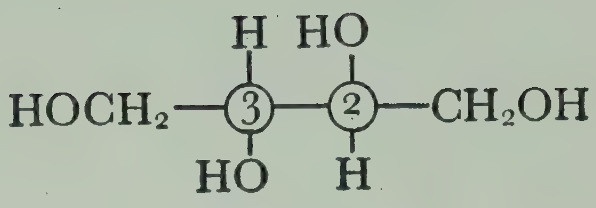
(XIXb)

(XXb)



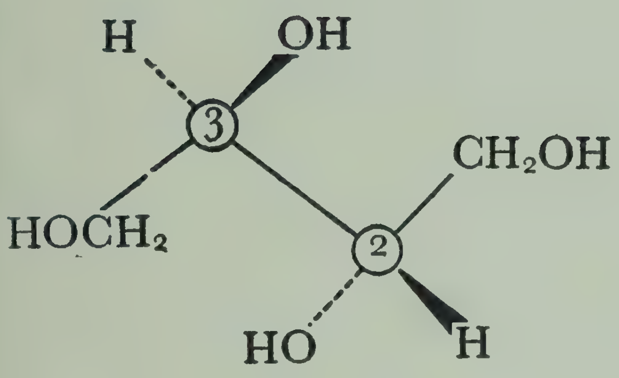
$$HOCH_2-\underset{HO}{\overset{H}{\textcircled{3}}}-\underset{HO}{\overset{H}{\textcircled{2}}}-CH_2OH$$

(XXII)

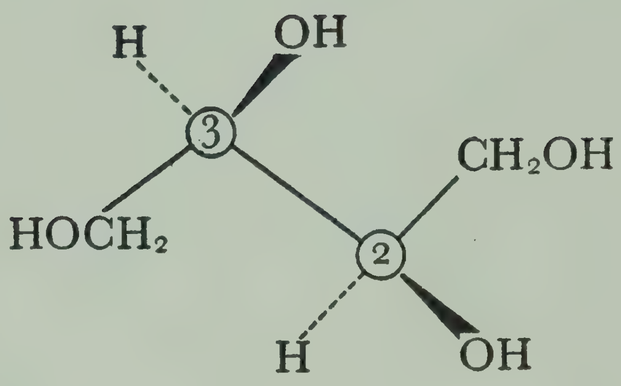


$$HOCH_2-\underset{HO}{\overset{H}{\textcircled{3}}}-\underset{H}{\overset{HO}{\textcircled{2}}}-CH_2OH$$

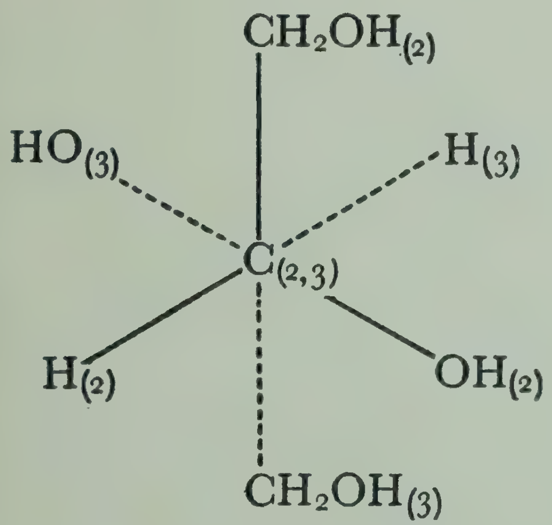
(XXIII)


H
OH
3
CH₂OH
HOCH₂
2
HO
H

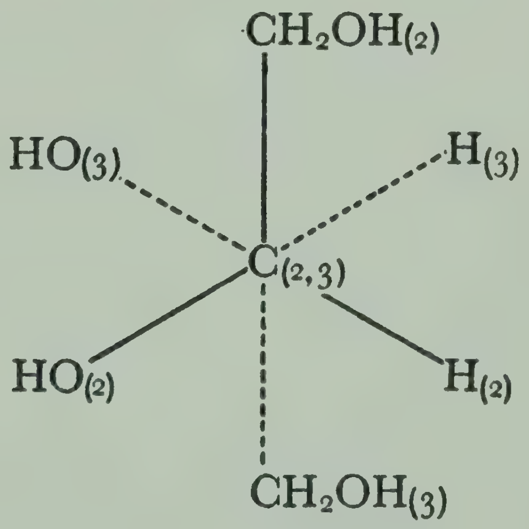
(XXIIa)


H
OH
3
CH₂OH
HOCH₂
2
H
OH

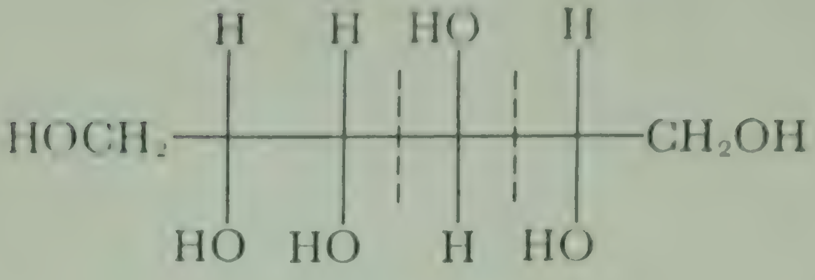
(XXIIIa)


CH₂OH(2)
HO(3)
H(3)
C(2,3)
H(2)
OH(2)
CH₂OH(3)

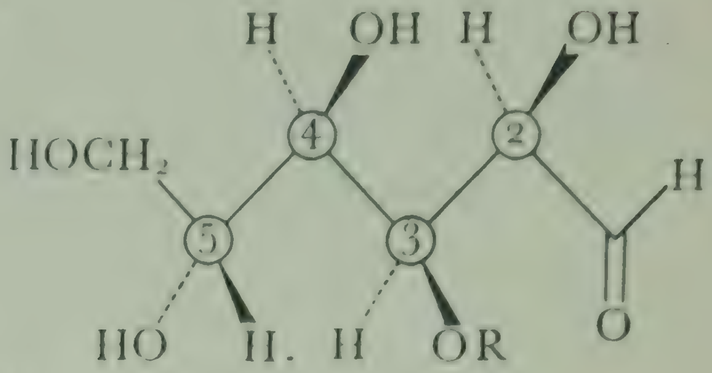
(XXIIb)


CH₂OH(2)
HO(3)
H(3)
C(2,3)
HO(2)
H(2)
CH₂OH(3)

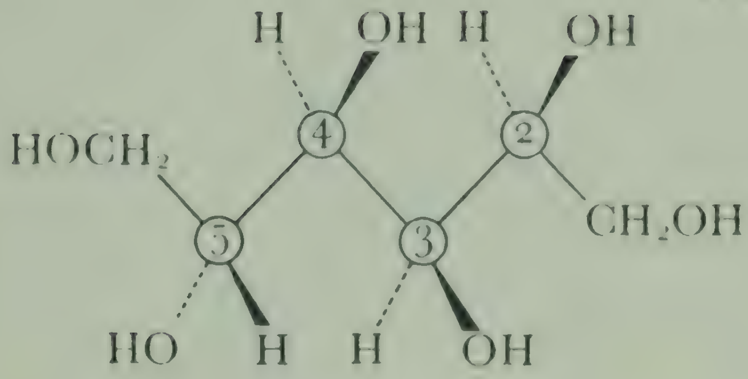
(XXIIIb)


H
H
HO
H
HOCH2
CH2OH
HO
HO
H
HO

(XXIV)

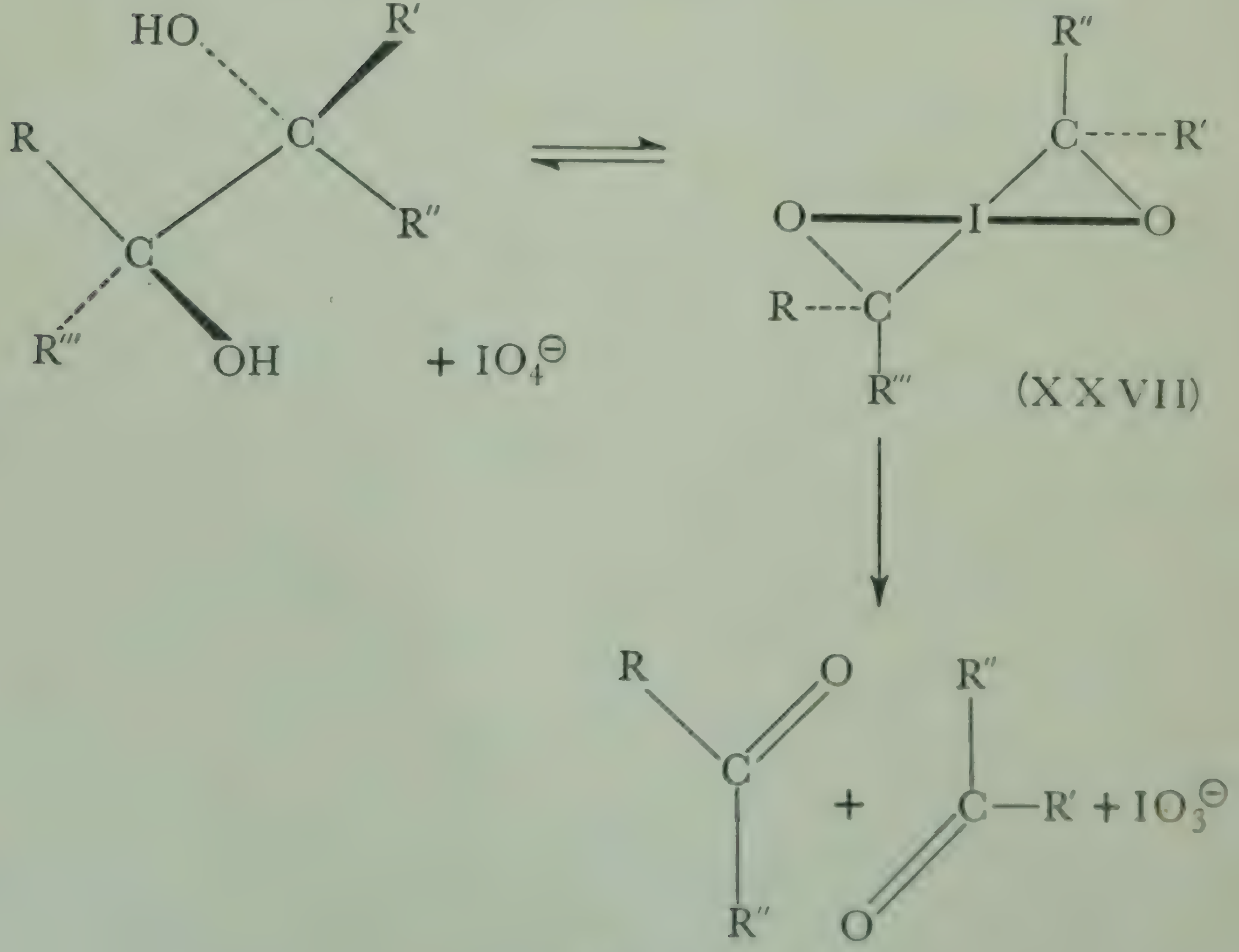

H
OH
H
OH
HOCH2
4
2
H
5
3
HO
H.
H
OR
O

(XXV)

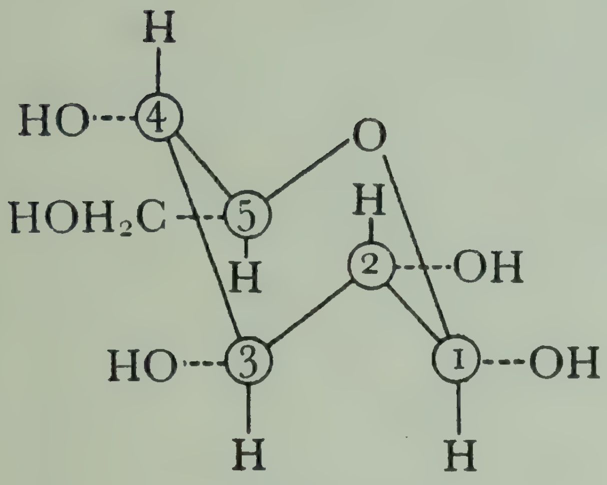

H
OH
H
OH
HOCH2
4
2
CH2OH
5
3
HO
H
H
OH

(XXVI)

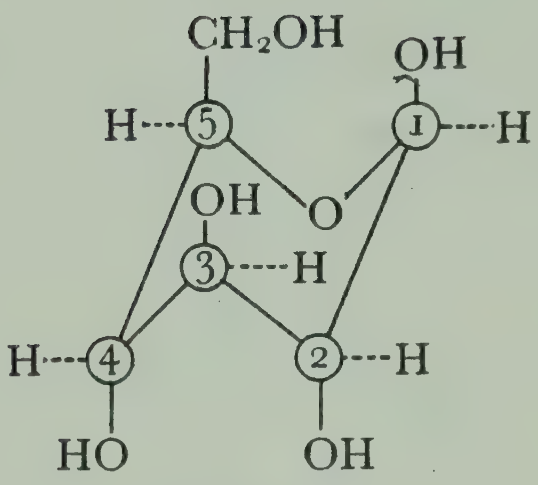
---- preferential attack by periodate


HO
R′
R
C
R″
C
R‴
OH
+ IO4⊖
R″
C
R′
O
I
O
R
C
R‴
R
O
C
R″
+
R″
C
R′
+ IO3⊖
O

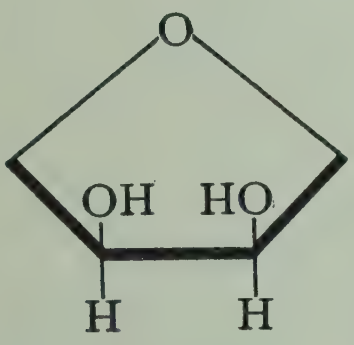
(XXVII)


H
HO
4
O
HOH$_2$C
5
H
H
2
OH
HO
3
1
OH
H
H

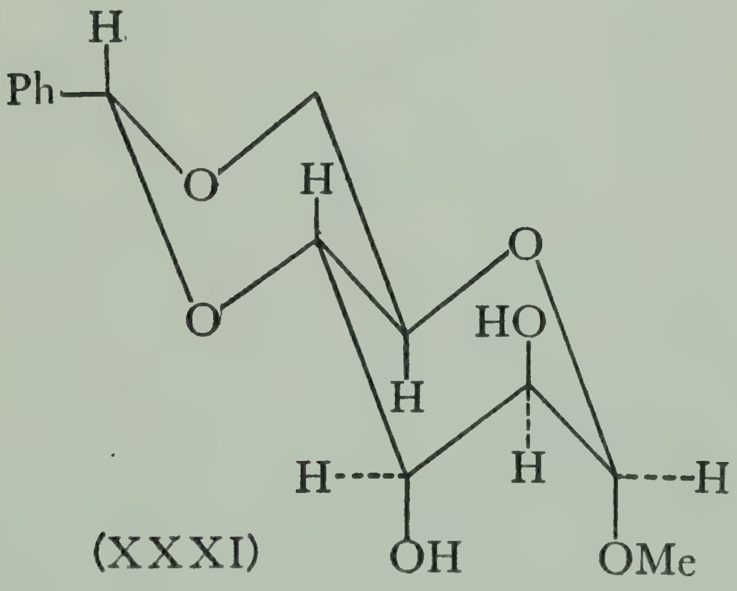
(XXVIII)


CH$_2$OH
OH
H
5
1
H
OH
O
3
H
H
4
2
H
HO
OH

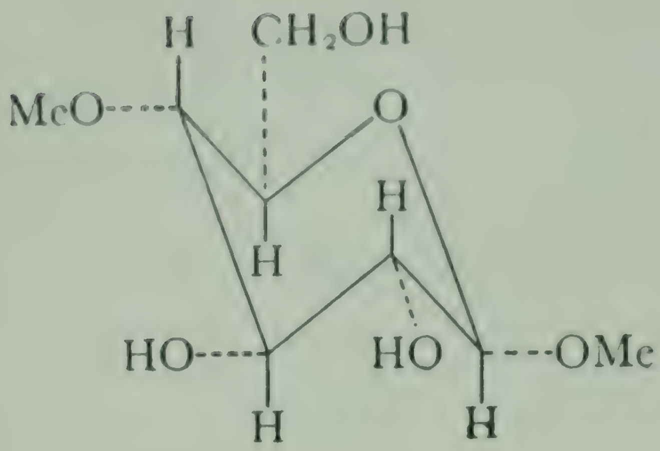
(XXIX)



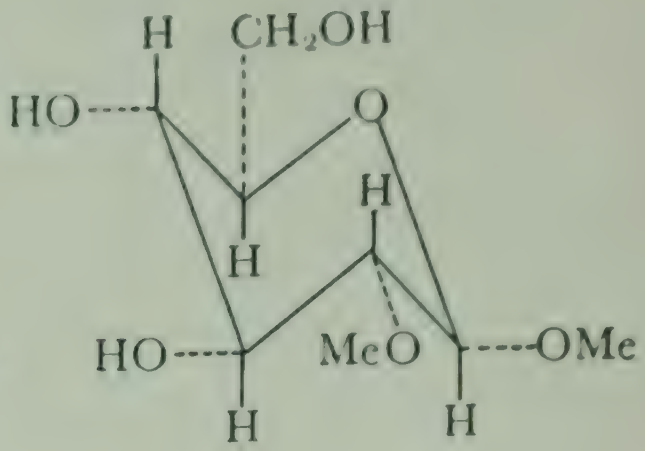
(XXX)


H
Ph
O
H
O
O
HO
H
H
H
H
OH
OMe

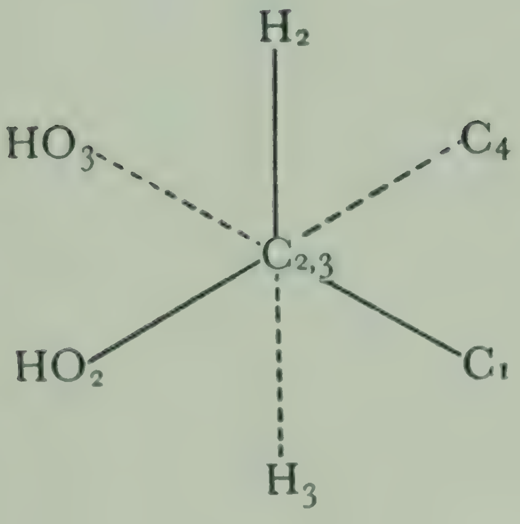
(XXXI)


H
CH₂OH
MeO
O
H
H
HO
HO
OMe
H
H

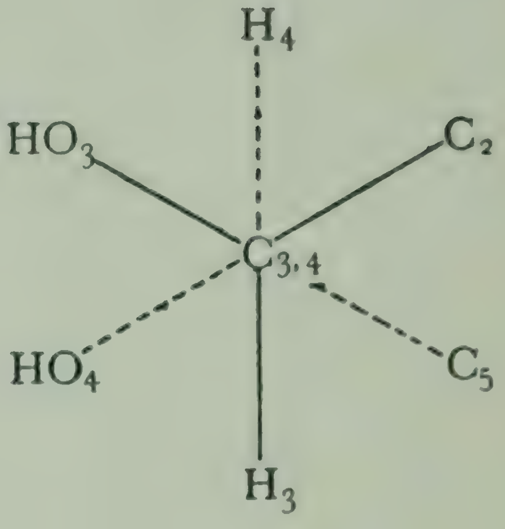
(XXXII)


H
CH₂OH
HO
O
H
H
HO
MeO
OMe
H
H

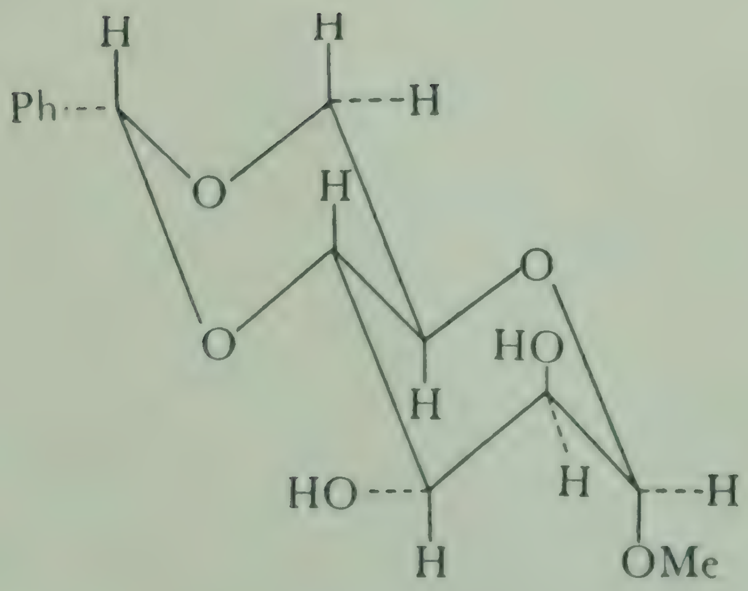
(XXXIII)


H₂
HO₃
C₄
C₂,₃
HO₂
C₁
H₃

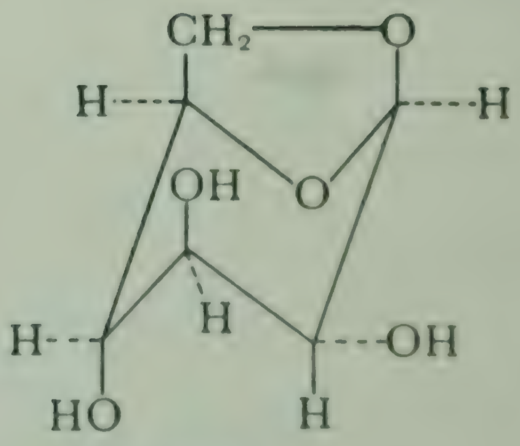
(XXXIIa)


H₄
HO₃
C₂
C₃,₄
HO₄
C₅
H₃

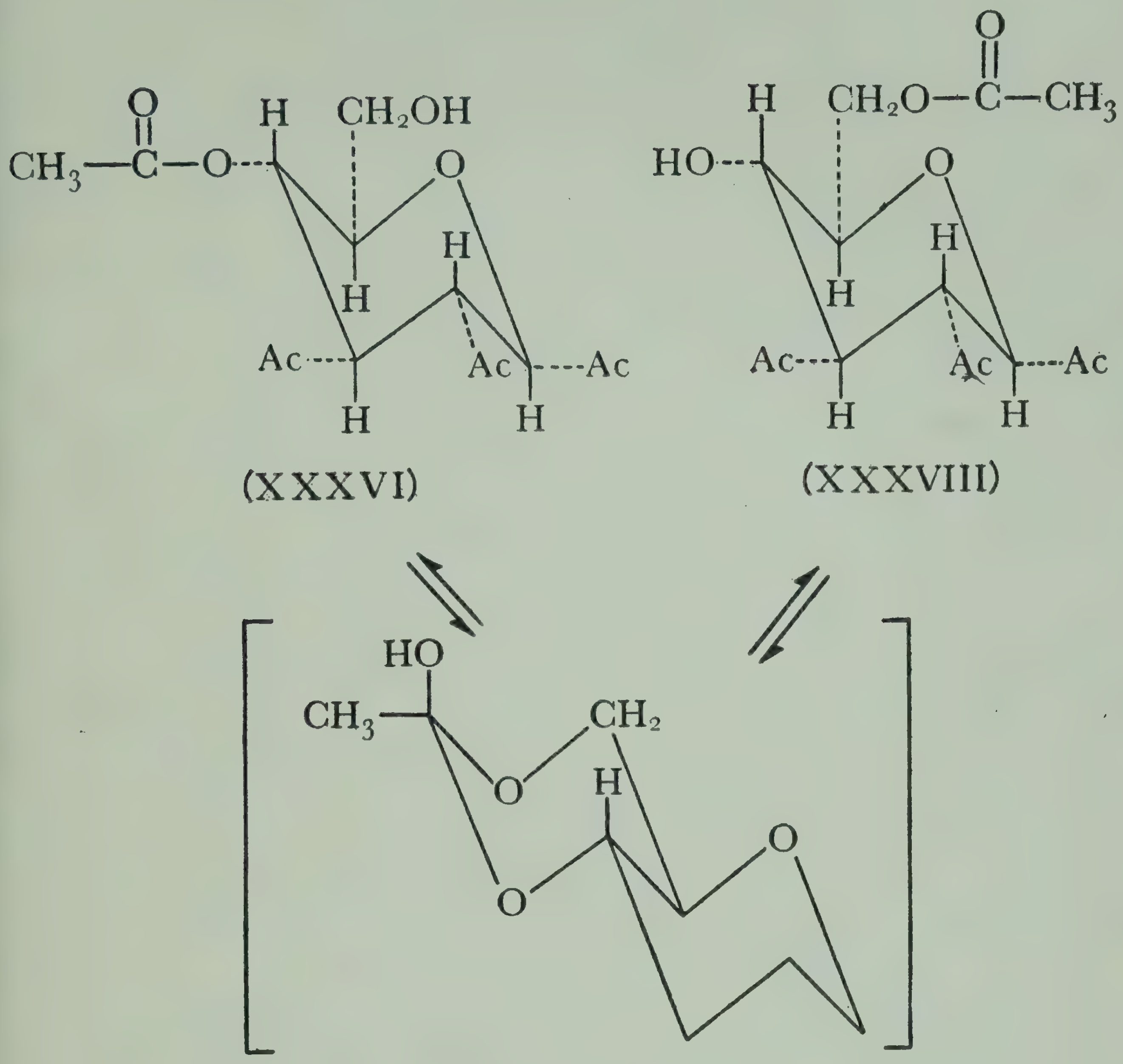
(XXXIIIa)


H
H
Ph
H
O
H
O
O
H
HO
HO
H
H
H
OMe

(XXXIV)


CH₂
O
H
H
OH
O
H
H
OH
HO
H

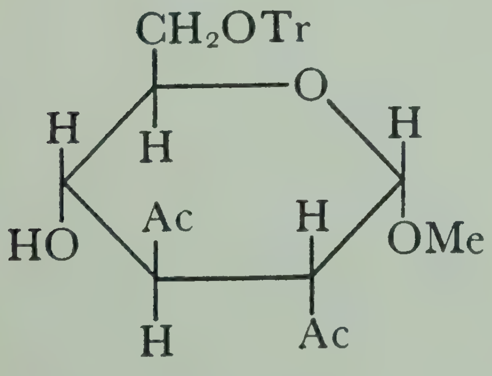
(XXXV)



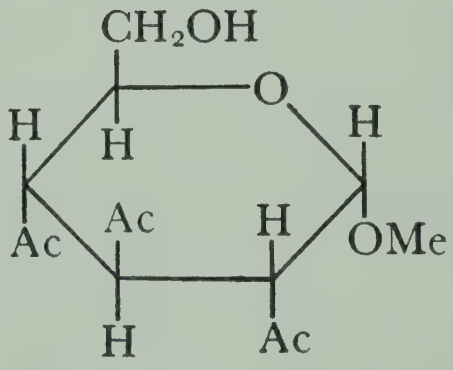
(XXXVI)

(XXXVIII)

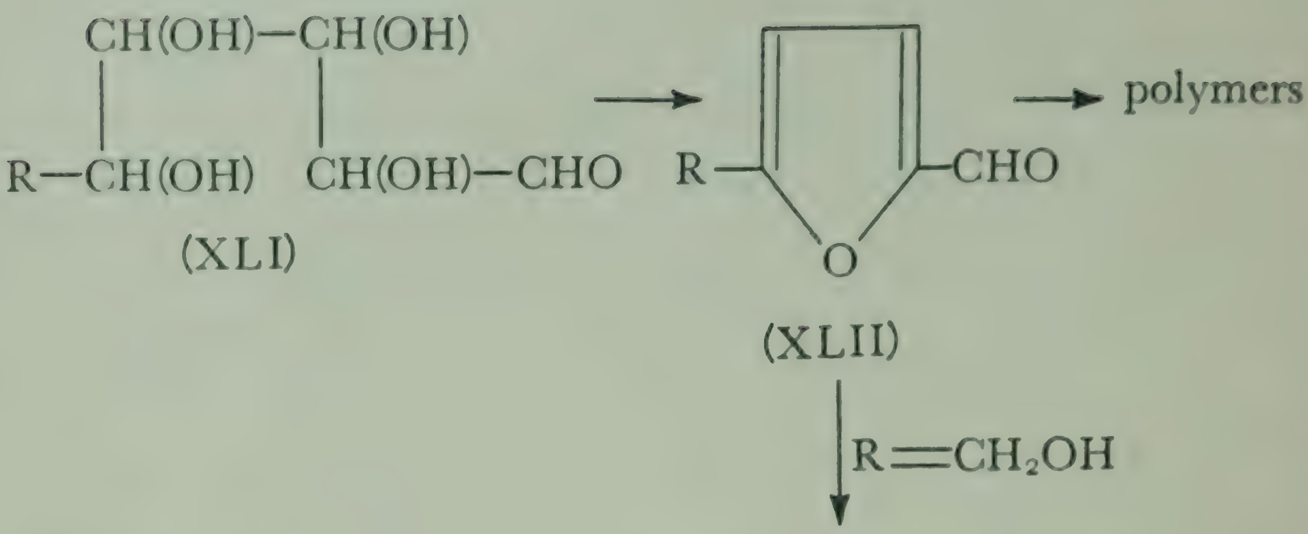
(XXXVII)



(XXXIX)

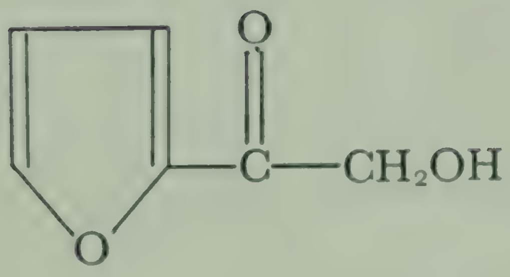


(XL)

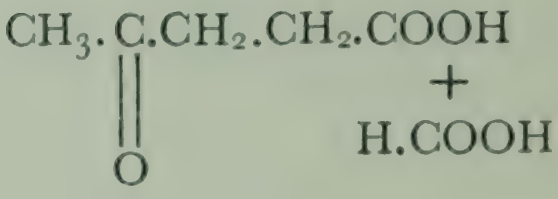

CH(OH)—CH(OH)
R—CH(OH)
CH(OH)—CHO
R
CHO
O
polymers
R=CH₂OH

(XLI)

(XLII)

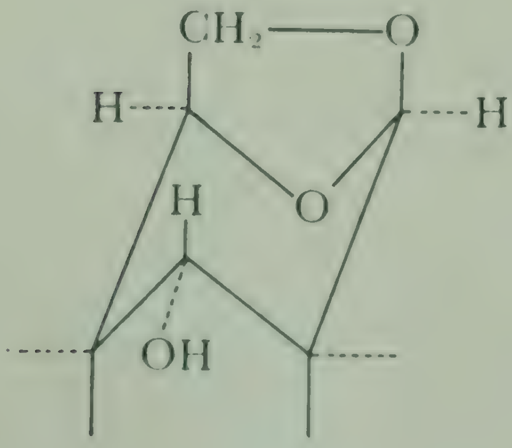


(XLIII)

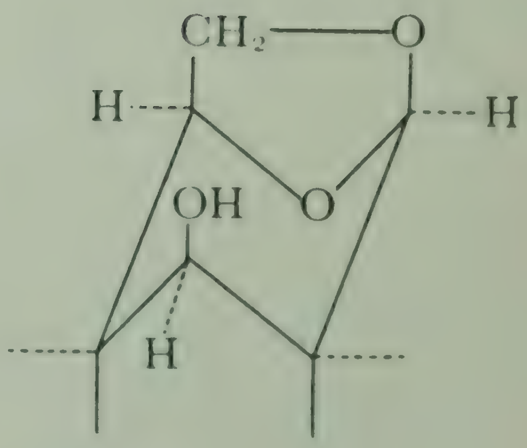


$$CH_3.\underset{\displaystyle\overset{\|}{O}}{C}.CH_2.CH_2.COOH + H.COOH$$

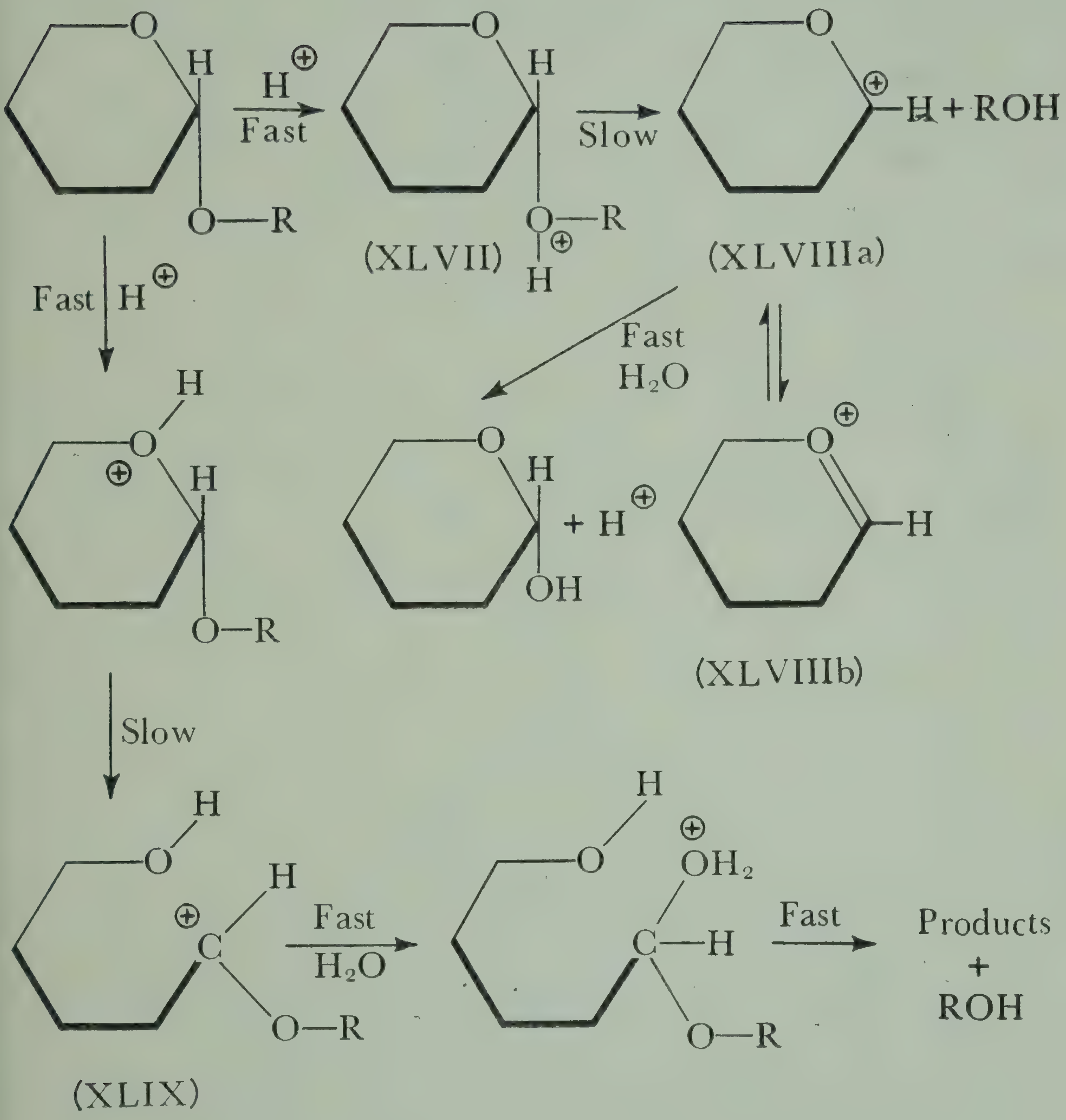
(XLIV)

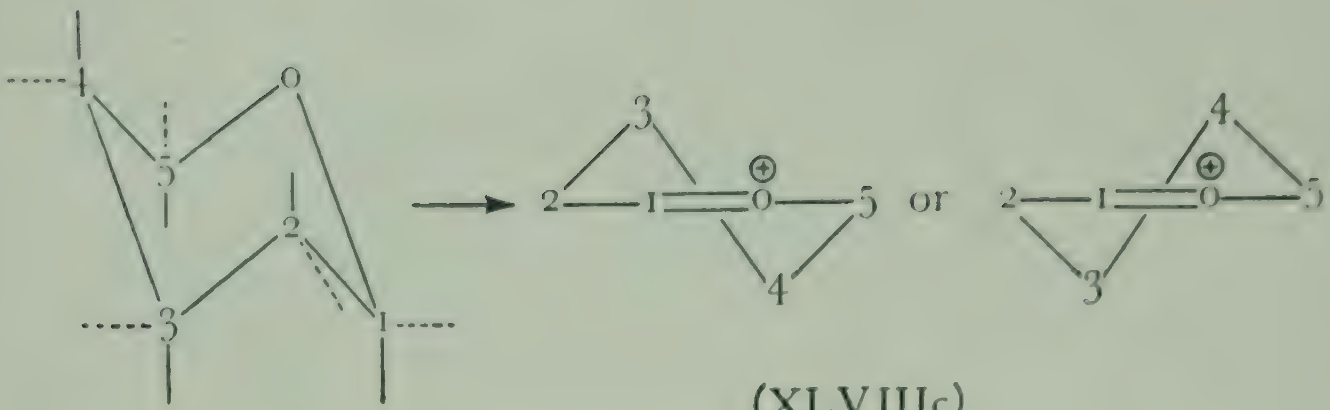

CH₂
O
H
H
H
O
OH

(XLV)


CH₂
O
H
H
OH
O
H

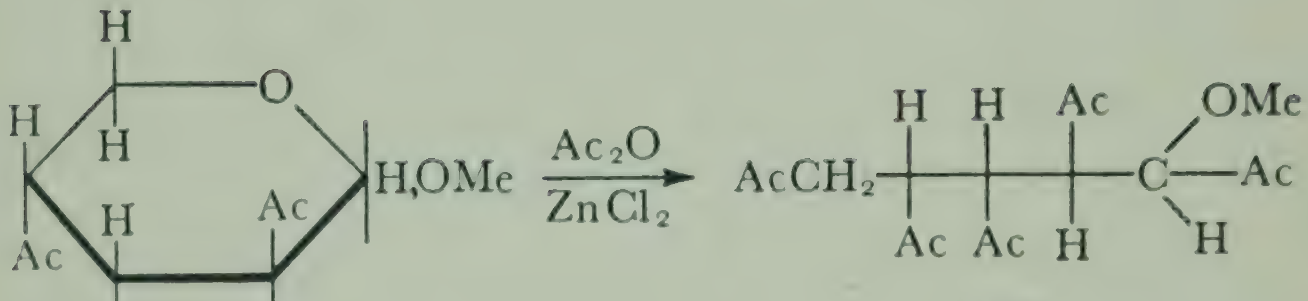
(XLVI)


Fast
Slow
(XLVII)
(XLVIIIa)
Fast
H_2O
(XLVIIIb)
Fast
Slow
(XLIX)
Fast
H_2O
Fast
Products
+
ROH


4
O
5
2
3
1
3
2
1
⊕
O
5
4
or
4
⊕
O
5
2
1
3

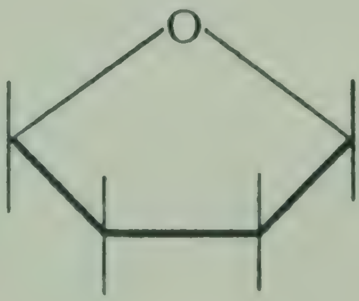
(LIII)

(XLVIIIc)

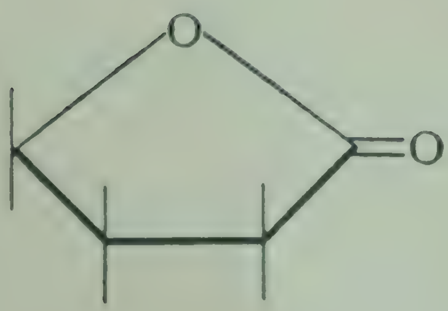

H
O
H
H
H,OMe
H
Ac
Ac
Ac
H
Ac₂O
ZnCl₂
H
H
Ac
OMe
AcCH₂
C
Ac
Ac
Ac
H
H

(L)

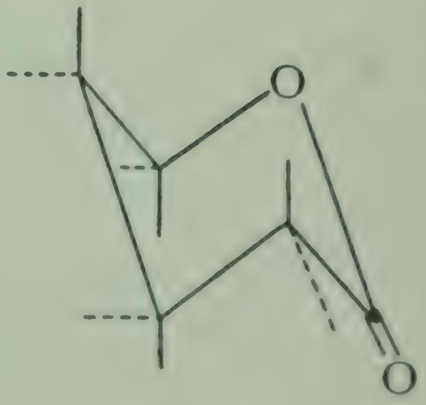
(LI)



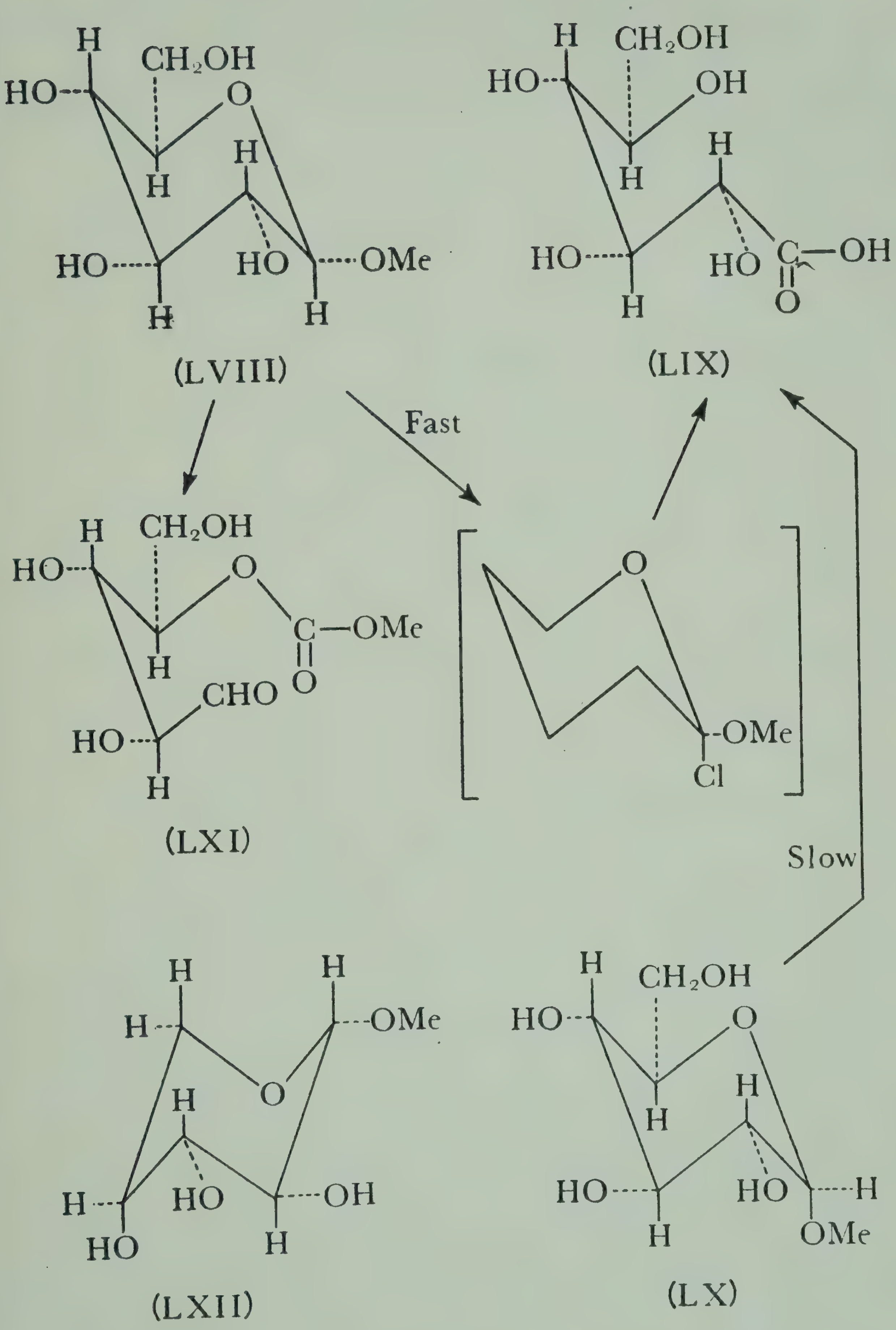
(LII)

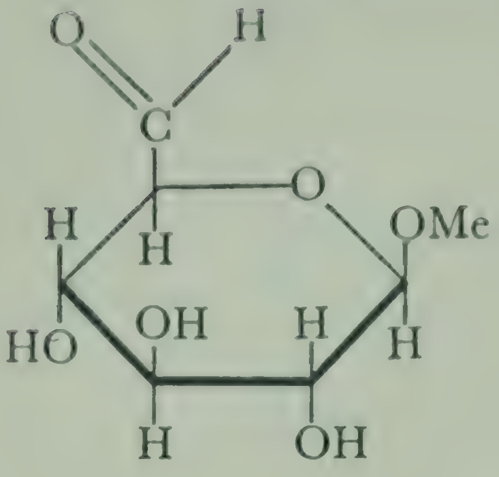


(LIV)

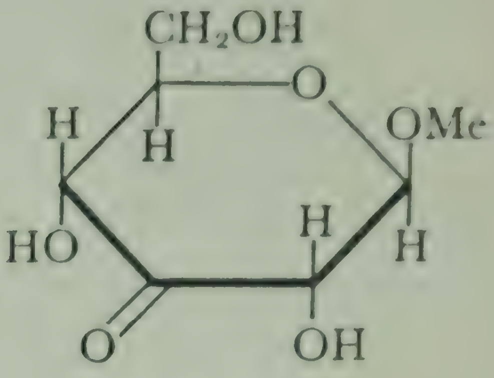


(LV)

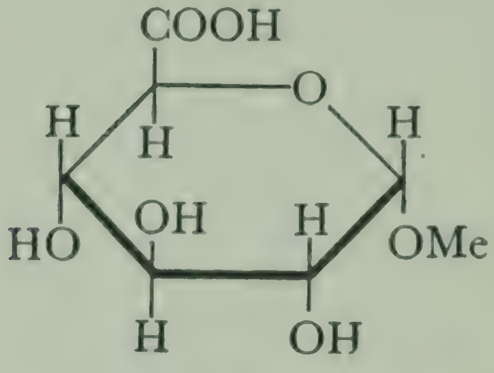

(LVIII)
Fast
(LIX)
(LXI)
Slow
(LXII)
(LX)



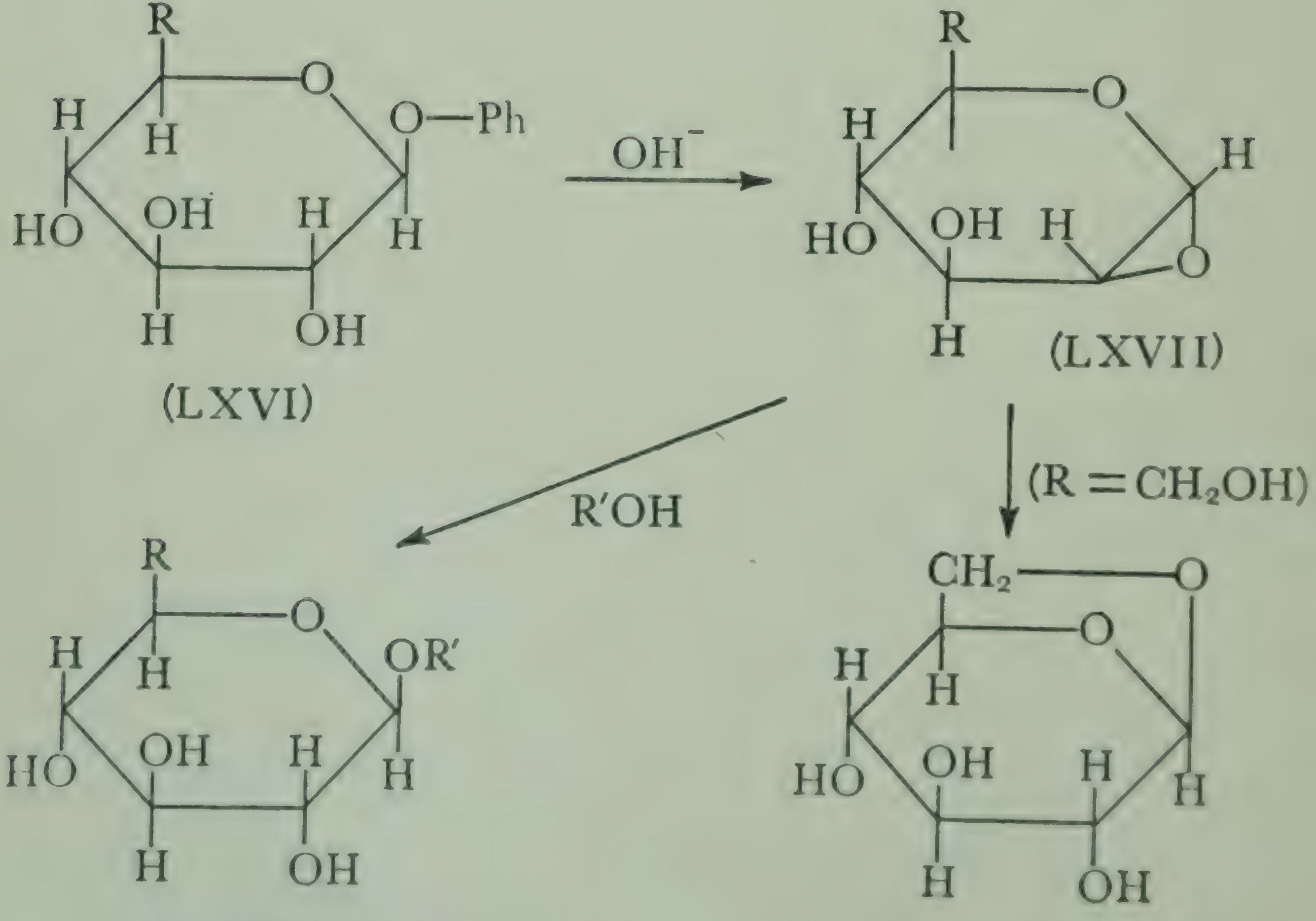
(LXIII)



(LXIV)



(LXV)



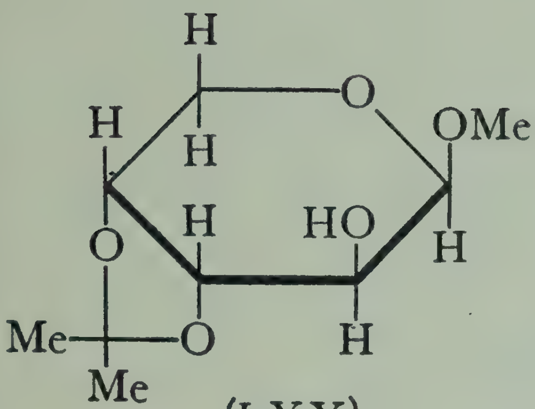
OH⁻
R'OH
(R = CH₂OH)

(LXVI)

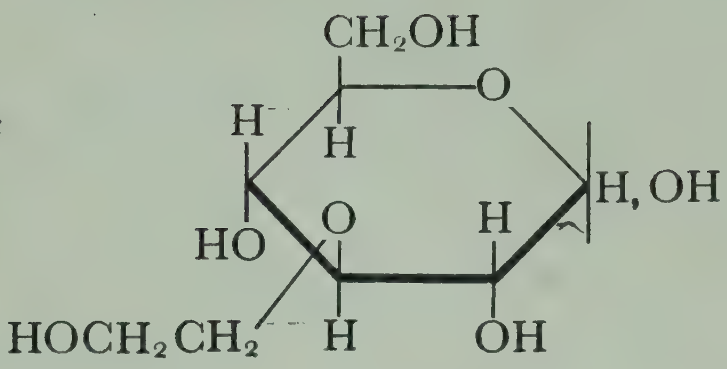
(LXVII)

(LXVIII)

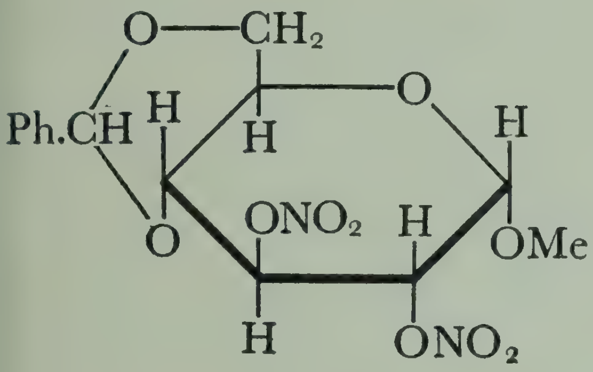
(LXIX)



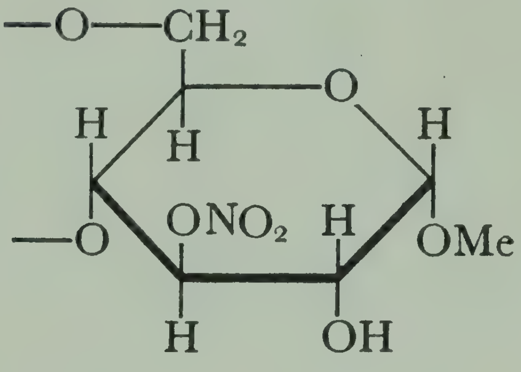
(LXX)



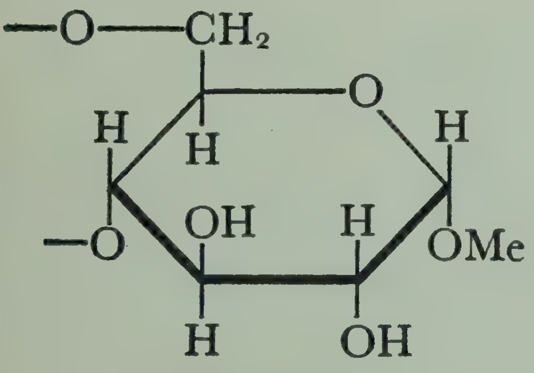
(LXXI)



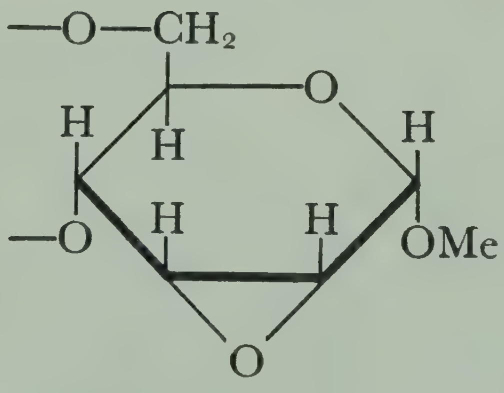
(LXXII)



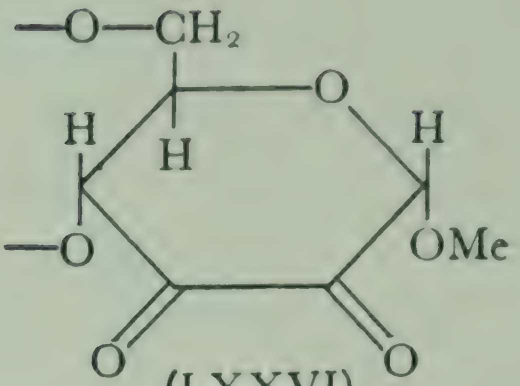
(LXXIII)



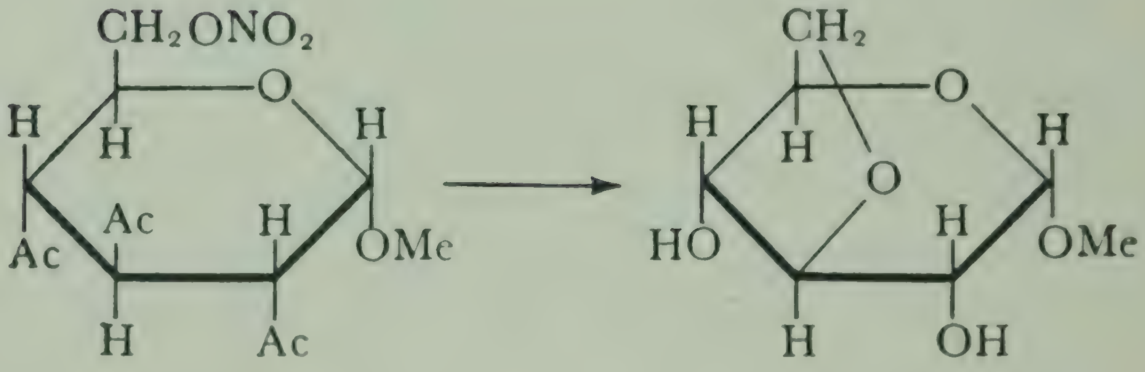
(LXXIV)



(LXXV)

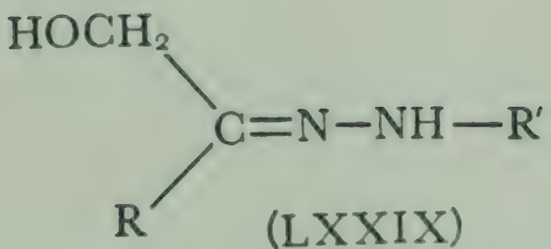


(LXXVI)



(LXXVII)

(LXXVIII)

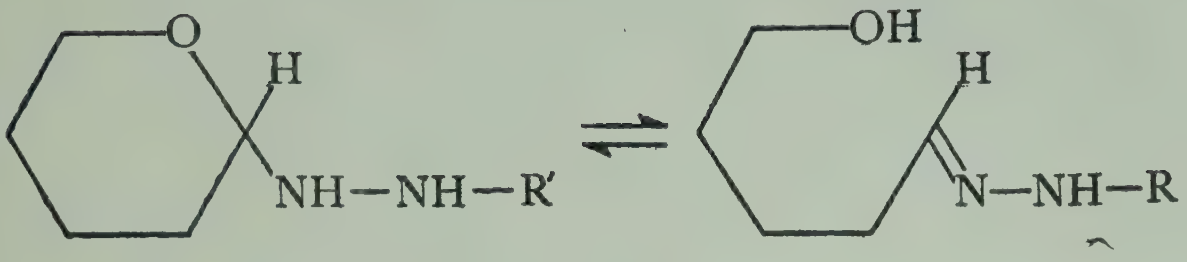


$$\begin{matrix} \mathrm{HOCH_2} & & \\ & \diagdown & \\ & & \mathrm{C{=}N{-}NH{-}R'} \\ & \diagup & \\ \mathrm{R} & & \end{matrix}$$

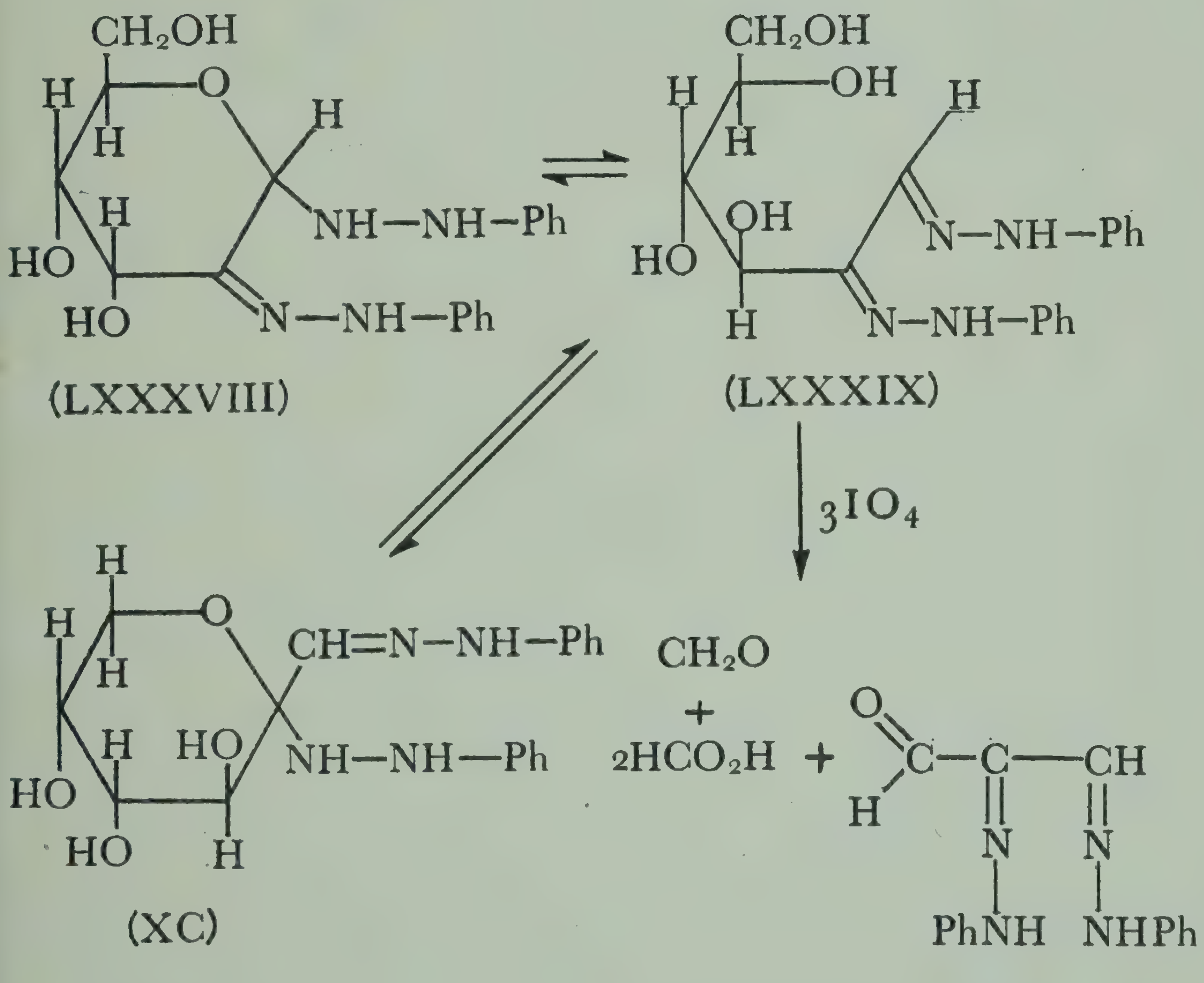
(LXXIX)

$R-CH=N-N-R'R'$

(LXXX)

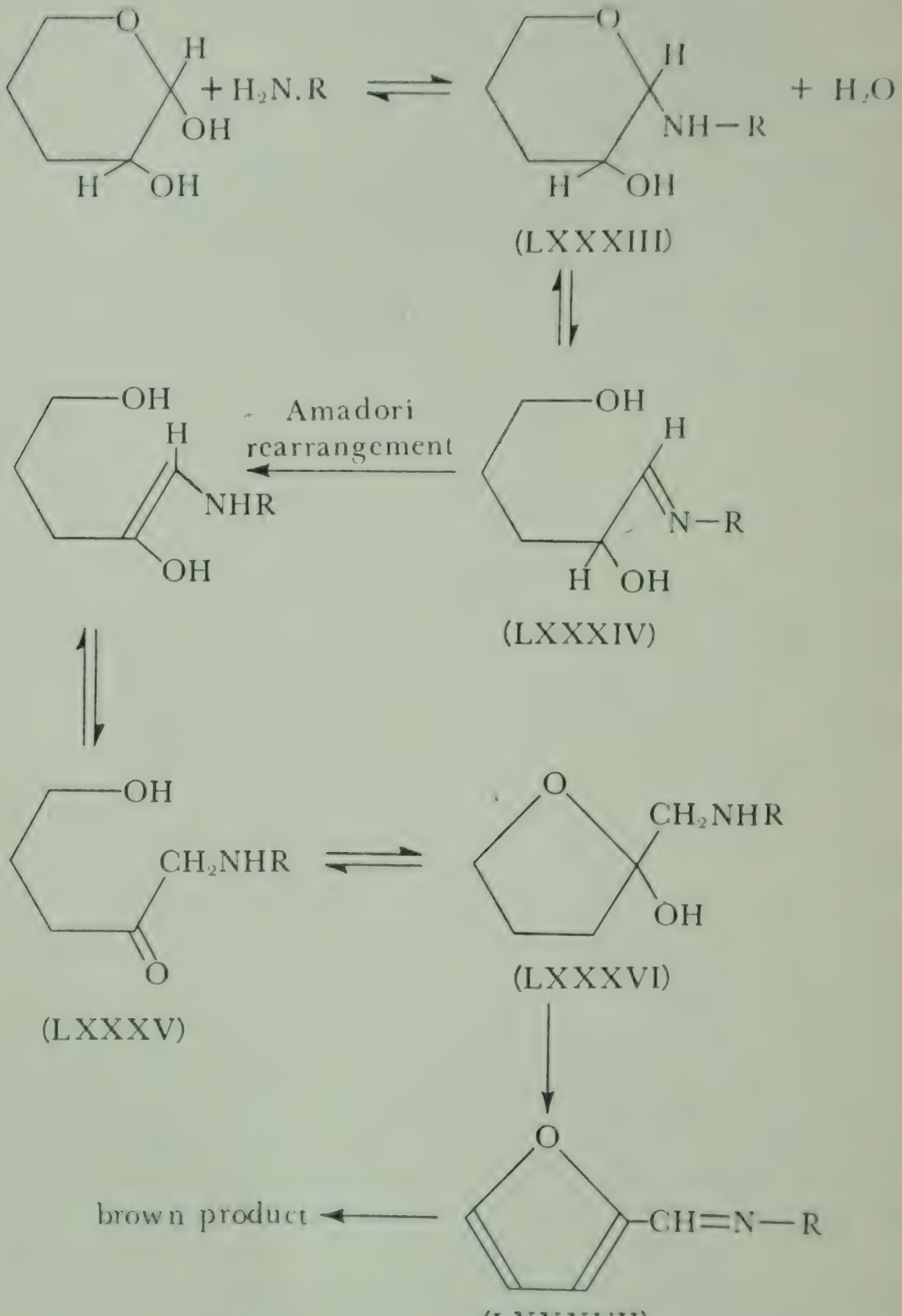

O
H
NH—NH—R′
OH
H
N—NH—R

(LXXXI) (LXXXII)


CH₂OH
H
O
H
H
H
HO
HO
NH—NH—Ph
N—NH—Ph
CH₂OH
OH
H
H
OH
HO
H
N—NH—Ph
N—NH—Ph
3IO₄
H
H
O
H
CH=N—NH—Ph
H HO
NH—NH—Ph
HO
HO H
CH₂O
+
2HCO₂H +
O
C—C—CH
H
N N
PhNH NHPh

(LXXXVIII) (LXXXIX)

(XC) (XCI)


O
H
+ H₂N.R
OH
H
OH
O
H
NH—R
+ H₂O
H
OH
OH
H
NHR
OH
Amadori rearrangement
OH
H
N—R
H
OH
OH
CH₂NHR
O
O
CH₂NHR
OH
O
brown product
CH=N—R

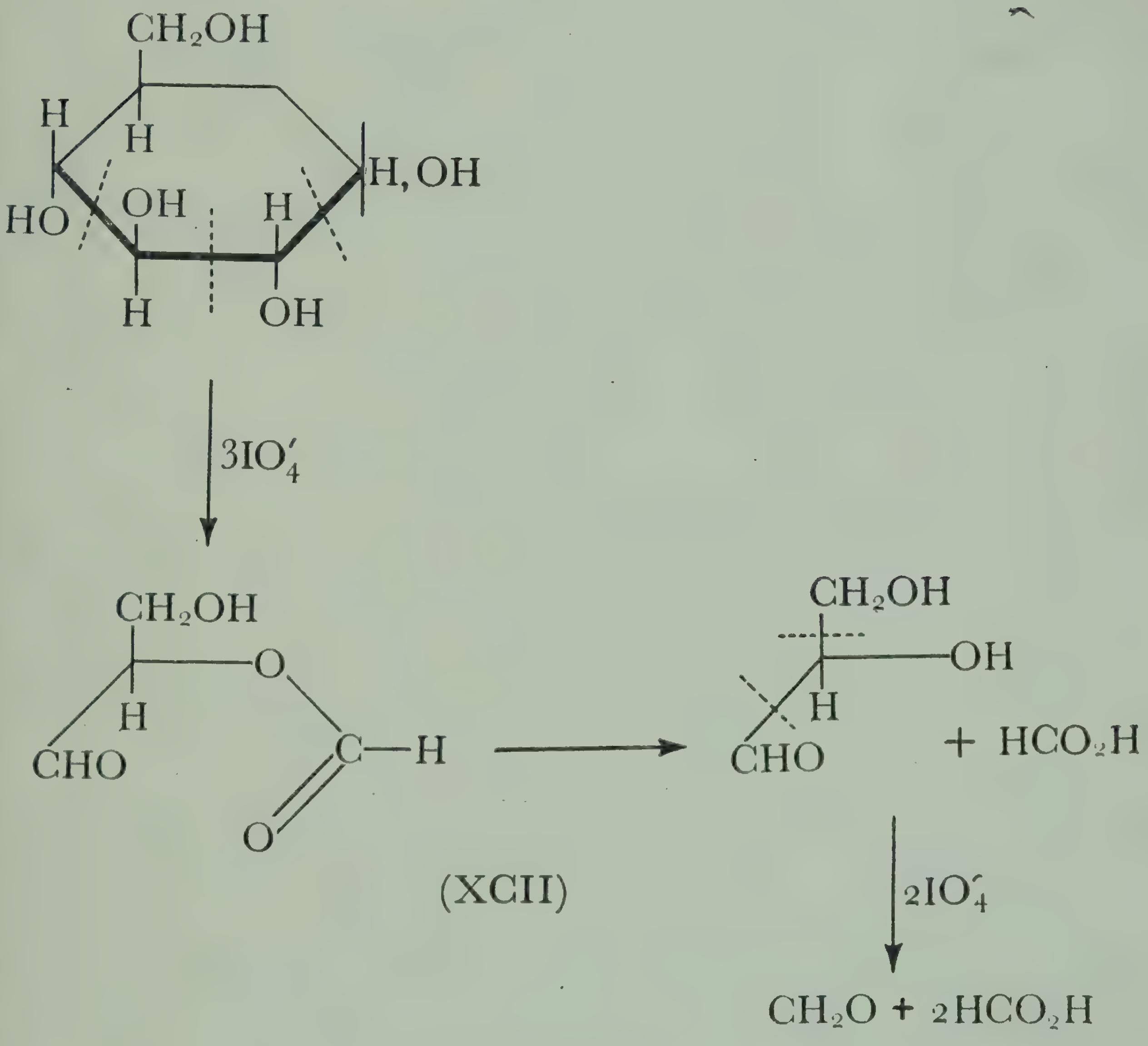
(LXXXIII)

(LXXXIV)

(LXXXV)

(LXXXVI)

(LXXXVII)


CH₂OH
H
H
H, OH
HO
OH
H
H
OH
3IO′₄
CH₂OH
O
H
CHO
C—H
O
CH₂OH
OH
H
CHO
+ HCO₂H
2IO′₄
CH₂O + 2HCO₂H

(XCII)

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2

MOLECULAR STRUCTURE OF CELLULOSE AND STARCH

INTRODUCTION

To elucidate the structure of a polysaccharide, the constituent sugars must be identified and their proportions determined quantitatively. Next, the positions of the linkages between the sugar units and the configurations of the anomeric carbon atoms have to be found. If the relative positions of the different sugars can be worked out and the average molecular weight determined then a complete picture of the ' average ' molecule will be available.

Cellulose and starch, two polysaccharides which occur abundantly in Nature, are built up, as far as is known, of D-glucose units only. This means that they are comparatively simple polymers and so a fairly complete knowledge of their molecular structure has been obtained. Many of the methods which have been used, and which will now be described, are of general application for determining polysaccharide structure.

GENERAL METHODS

Degradation of Cellulose and Starch to D-Glucose

One of the first steps in structural studies on cellulose and starch was to show that, when hydrolysed, they yielded only D-glucose, which could be isolated in quantitative yield. For starch, this was demonstrated by hydrolysis of the polysaccharide with dilute acid when crystalline D-glucose was isolated in high yield.[1] Also, through alcoholysis,[2] crystalline methyl D-glucose was obtained in 95 per cent of the theoretical yield, and from hydrolysis of methylated starch, methylated D-glucose derivatives were recovered in yields accounting for 97 per cent of the starch.[3] For cellulose, the method most successfully used was acetolysis with chlorine and sulphur dioxide as catalysts to give highly degraded acetates which were then subjected to methanolysis. This simultaneously removed the acetyl groups and further degraded the cellulose to a crystalline mixture of methyl α- and β-D-glucopyranosides, obtained in a yield accounting for 95·5 per cent of the cellulose.[4]

Methylation

The methylation technique used by Haworth to elucidate the structures of disaccharides has been applied in polysaccharide studies. It involves complete methylation, using dimethyl sulphate–sodium hydroxide followed, if necessary, by methyl iodide–silver oxide, then hydrolysis, and finally separation and identification of the methylated sugars (see Figure 1). The positions occupied by the methyl groups in the fragments correspond with those of the unsubstituted hydroxyl groups in the original polysaccharide. The method is somewhat limited however, particularly when applied to more complex polysaccharides, since it does not establish the order of the sugar units or the configuration of the anomeric carbon atom. Nor does it distinguish between, for example, a pyranose sugar linked through $C_{(4)}$ and a furanose sugar linked through $C_{(5)}$.

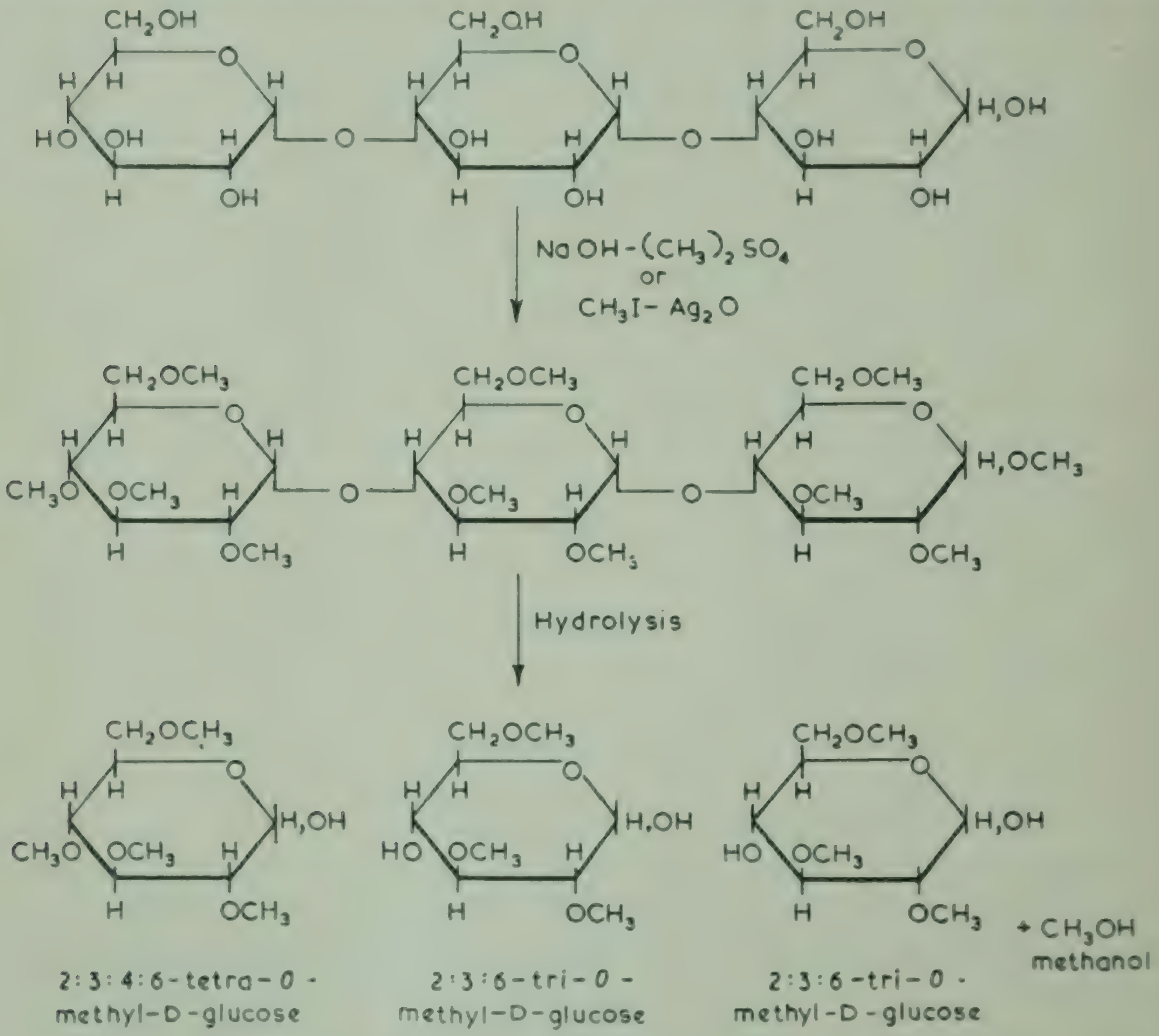
NaOH-(CH3)2SO4
or
CH3I- Ag2O
Hydrolysis
+ CH3OH
methanol
2:3:4:6-tetra-O-methyl-D-glucose
2:3:6-tri-O-methyl-D-glucose
2:3:6-tri-O-methyl-D-glucose

Figure 1.

When this technique was applied to either cellulose[5–10] or starch,[11–16] the main product was 2 : 3 : 6-tri-*O*-methyl-α-D-glucopyranose, confirming their constitutions as long-chain polymers. In addition, smaller amounts of tetra- and di-methyl ethers of α-D-glucopyranose were found in the hydrolysates of the methylated products.[5,12–18] The tetra-*O*-methyl-α-D-glucose must have been derived from non-reducing chain-ends, whereas the di-*O*-methyl-α-D-glucose might have arisen through incomplete methylation or from glucose units involved in branching. On this basis the picture which emerged was that both starch and cellulose are composed of terminating chains of glucose units, the minimum average length of the chains being 25 glucose units for starch and 100–200 for cellulose. Recent work has indicated, however, that chain degradation occurs during methylation, even under carefully controlled conditions. This makes methylation rather unreliable as a means of end-group assay, particularly for long, unbranched chains.[19–22]

Partial Hydrolysis

Partial hydrolysis to give scission products intermediate in complexity between the monosaccharide units and the polysaccharide is a very useful method of supplementing information obtained from methylation. Recent developments in chromatographic techniques, which facilitate the separation and identification of the products of partial hydrolysis, make the method of increasing importance in structural determinations.

Cellulose can be degraded to cellobiose octa-acetate in a yield of 50–51 per cent, using a mixture of acetic acid, acetic anhydride, and sulphuric acid,[23] while under less drastic conditions a series of oligosaccharides from cellotriose to celloheptaose have been identified in the hydrolysate.[24–27] The structure of cellobiose was established as 4-*O*-β-D-glucopyranosyl-D-glucopyranose.[28–31] Its recovery from cellulose was taken as evidence of the extensive existence of β-D-Gp 1—4 D-Gp in the polysaccharide. No linkage other than this was detected in any of the oligosaccharides.

Similarly, starch was degraded into hepta-*O*-acetyl maltosyl bromide and methyl octa-*O*-methyl maltobionate in considerable yields,[32,12] and it was concluded that the main interglucose linkage in starch was the maltose one. Maltose was shown to be 4-*O*-α-D-glucopyranosyl-D-glucopyranose,[11,33–35] and it followed that the linkages in starch were also mainly α-D-Gp 1—4 D-Gp. A series of linear dextrins from maltose to maltoheptaose have also been isolated by partial hydrolysis of starch with sulphuric acid, followed by fractionation of the products on a column of charcoal–Celite.[36]

FOOD RESEARCH
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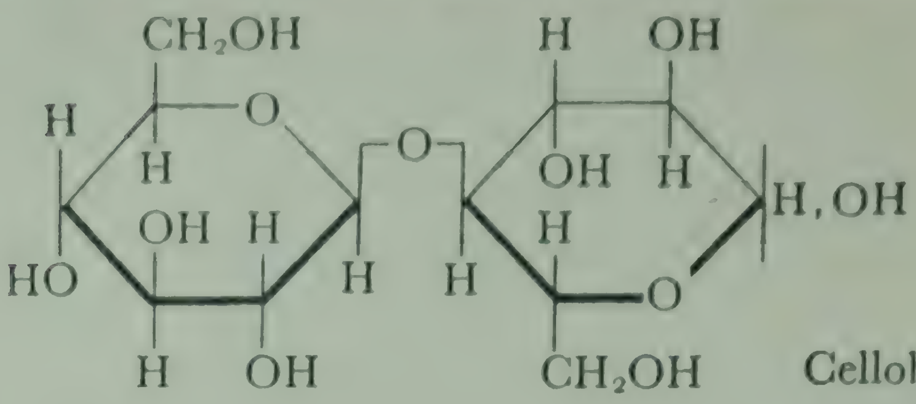
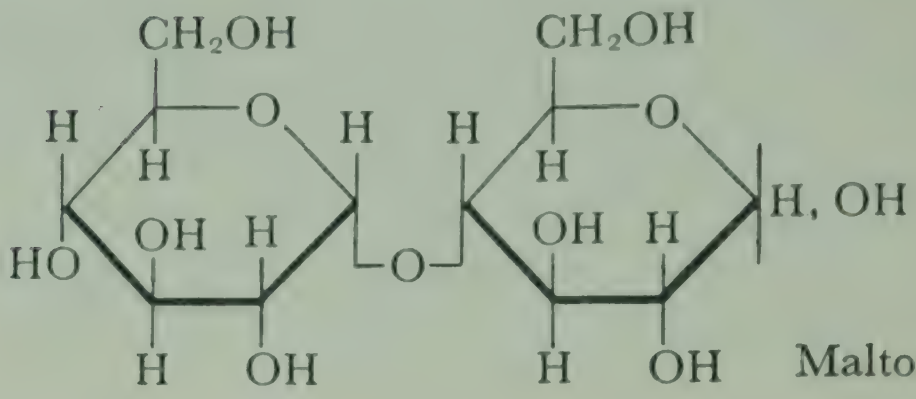
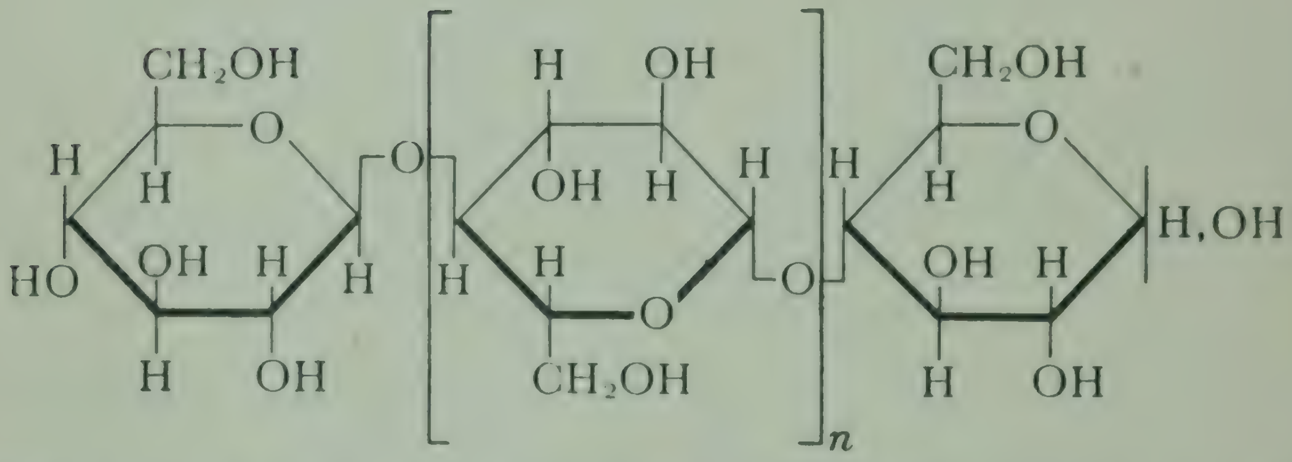


Cellobiose



Maltose



Cellotriose, $n = 1$; Cellotetraose, $n = 2$; Cellopentaose, $n = 3$; Cellohexaose, $n = 4$; Celloheptaose, $n = 5$.

Periodate Oxidation

The periodate oxidation of α-glycols, first noted[37] by Malaprade in 1928, has been widely used and developed for the elucidation of carbohydrate structures. It is particularly suited to studies of the more sensitive carbohydrates since the oxidation proceeds, under mild conditions, with a high degree of specificity.[38–40] Precise analytical procedures, including micro-scale methods, have been developed to follow the course of the reaction and estimate the oxidation products.[41–43]

Measurement of the formic acid produced during periodate oxidation is of particular value. In each molecule of a linear polymer made up of D-glucose units linked from $C_{(1)}$ to $C_{(4)}$, the non-reducing chain-end will give rise to one molecule of formic acid and the reducing chain-end to two. No formic acid will be produced from intermediate chain units. Measurement of the formic acid liberated will therefore give a measure of the average chain length. However, the production of formic acid at the reducing end of the molecule will be controlled by the rate of hydrolysis of the formate ester produced. A method of measuring chain length has been described in which the formic acid produced by hydrolysis of the formate ester is estimated[44] (see Figure 2).

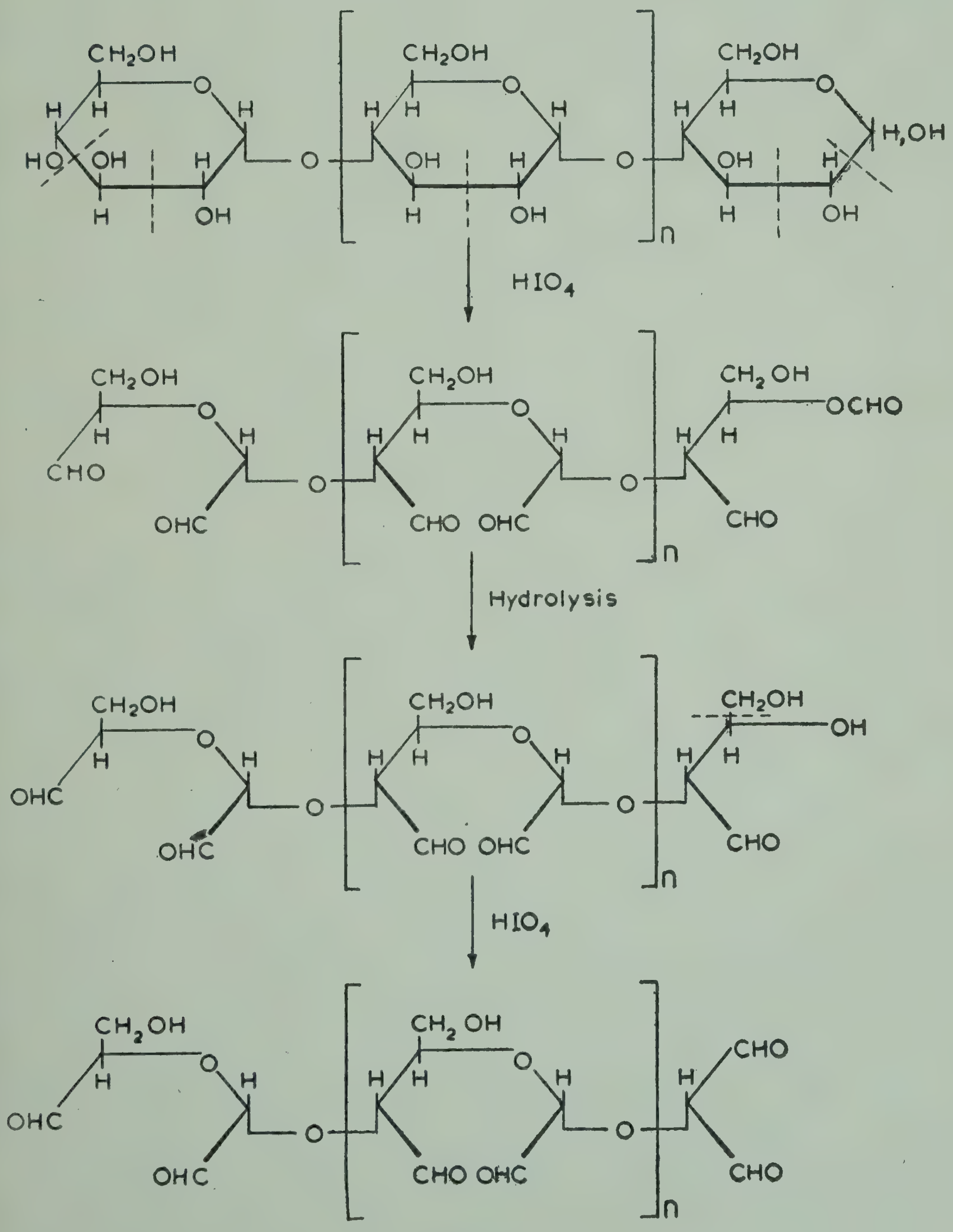


Figure 2.

In highly branched polysaccharides, where the number of reducing ends is small compared with the non-reducing ends, the yield of formic acid produced is a measure of the average number of polymerized units in the branch chains. On the other hand, in a linear polymer made up of D-glucopyranose units linked from $C_{(1)}$ to $C_{(3)}$, there is no α-glycol unit in any intermediate unit, and the periodate uptake itself will give a measure of chain length. In this case, hydrolysis of the oxidation product should yield large amounts of D-glucose and, in fact, the isolation of,[45–48] or failure to isolate,[47,49,50] D-glucose from oxidized, hydrolysed polysaccharides has been used as indicative of the absence or presence of glycol groupings.

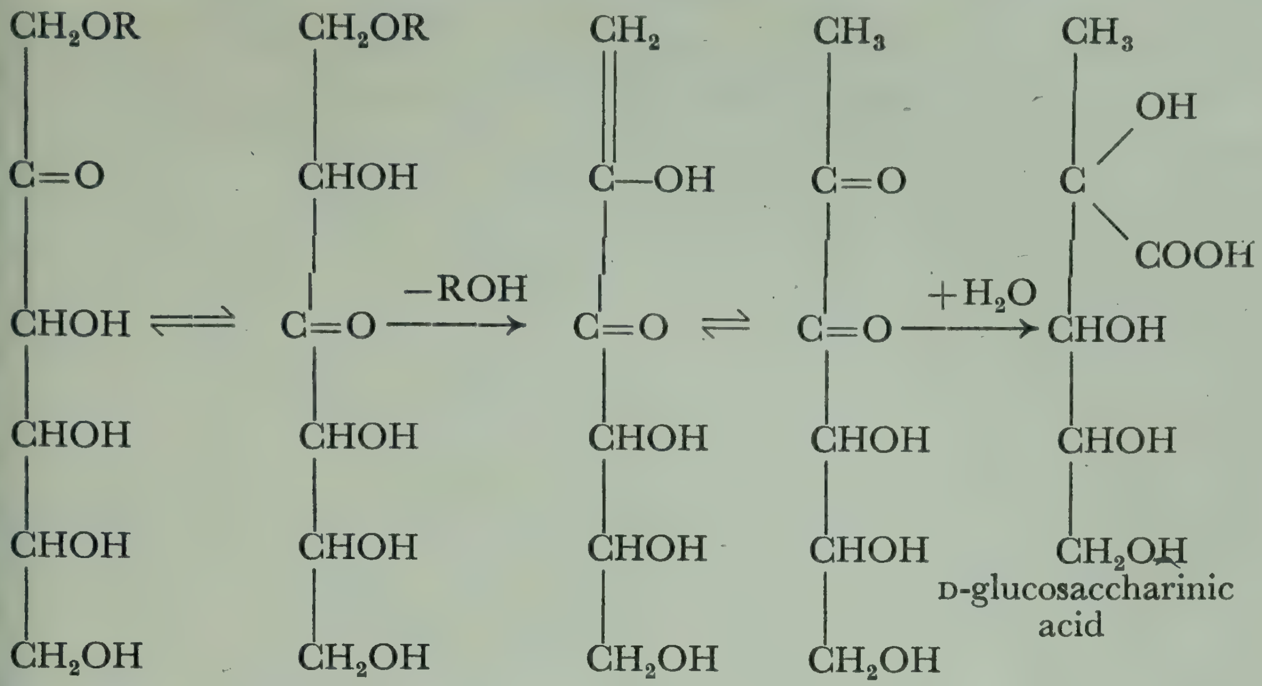
Certain difficulties may be encountered, however, particularly ' over-oxidation ', and an increasing number of non-specific and anomalous oxidations which must be borne in mind when the results obtained are interpreted in terms of structures are being reported.[51–56] For example, periodate oxidation will not stop abruptly at the stage shown in Figure 2 but will proceed further, with liberation of formic acid, giving rise to step-wise degradation of the chain from the reducing end. The effects of such ' over-oxidation ', which are discussed more fully in *The Hydrolytic and Oxidative Degradation of Cellulose* (p. 99), will be particularly important when estimation of the formic acid produced is used as a measure of the chain length of linear polysaccharides.

Step-wise Degradation

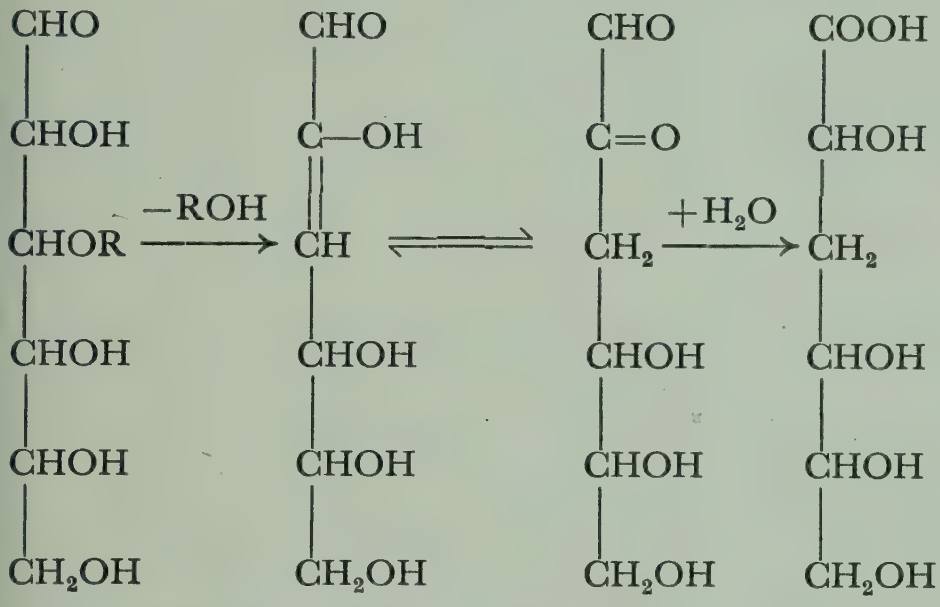
From the reducing end of the polysaccharide. In recent years, Kenner and his co-workers[57–67] have carried out extensive studies into the mechanism of alkaline degradation of carbohydrates. Their results indicate that the formation of D-saccharinic acid, or the *meta* or *iso* types of isomers, is diagnostic of 1-, 3-, and 4-*O*- substituted hexoses respectively (see Figure 3).

In accordance with this theory, alkaline degradation of maltose[60] and cellobiose[62] was found to yield D-gluco*iso*saccharinic acid and D-glucose, laminaribiose and turanose gave D-gluco*meta*saccharinic acid and D-glucose,[61] and lactose gave D-gluco*iso*saccharinic acid and D-galactose.[59] Studies have also been extended to the larger molecules of cellotetraose and laminarin where it is found that alkaline degradation proceeds by a ' peeling ' process from the reducing end of the chains.[62] Fuller details of this and other recent work on the alkaline degradation of cellulose, hydrocellulose, and oxycelluloses, are given in *The Alkaline Degradation of Cellulose and Chemically Modified Cellulose* (p. 106).

Corbett, Kenner, and Richards[63] point out that this type of

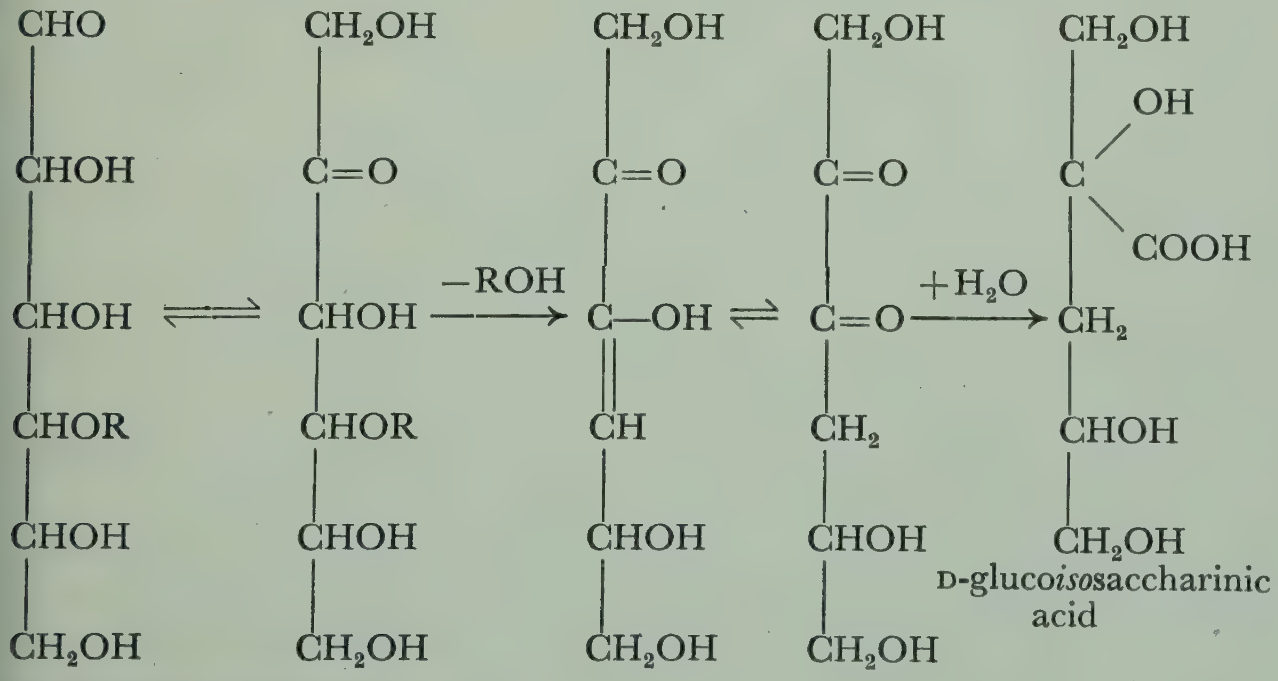

−ROH
+H₂O
D-glucosaccharinic acid

e.g. 1-*O*-methyl-D-fructose



e.g. 3-*O*-methyl-D-glucose

D-gluco*meta*saccharinic acid


−ROH
+H₂O
D-gluco*iso*saccharinic acid

e.g. 4-*O*-methyl-D-glucose

Figure 3.

reaction may be applied generally to structural studies on carbohydrates to give information on chain linkages and ring forms of the sugar units. Furthermore, the same type of reaction will apply to acids derived from monoses other than glucose so that the saccharinic acids produced by alkaline degradation will act as reference compounds.

From the Non-reducing End of the Polysaccharide

Barry and Dillon[68] have developed a step-wise degradation of polysaccharides made up of D-glucopyranose units linked together from $C_{(1)}$ to $C_{(3)}$. It depends on the intermediate units of such polymers being unattacked by periodate, and the method involves periodate oxidation, followed by treatment of the product with phenylhydrazine acetate. This splits the glycosidic linkage of the non-reducing terminal unit with separation of glyoxal diphenylhydrazone; the polysaccharide, one unit shorter, is recovered. The process may then be repeated and is useful in detecting linkages other than D-Gp 1—3 D-Gp in this type of polymer (see Figure 4).

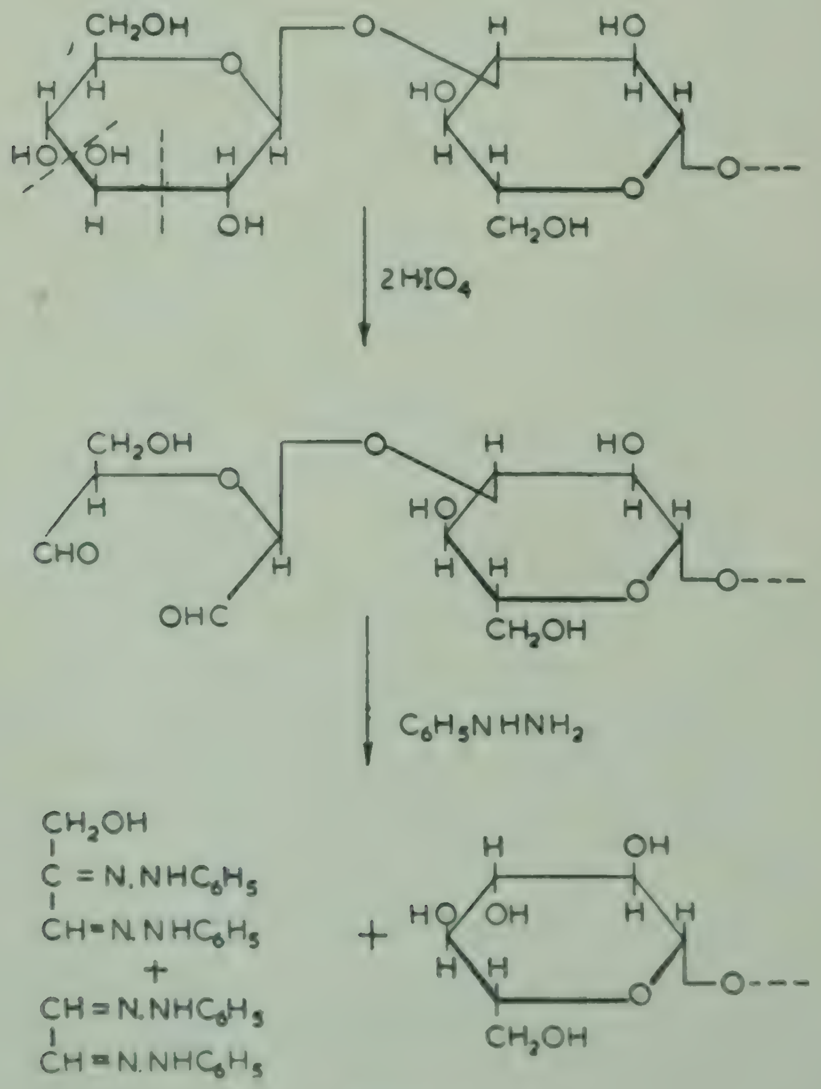


Figure 4.

CELLULOSE

Occurrence and Isolation

Cellulose forms the major portion of the cell walls of the higher plants, and is the basic constituent of plant life. Its chief sources are seed hairs, such as cotton; bast fibres, occurring in the inner bark of certain fibrous plants such as flax, jute, and ramie; leaf fibres, such as hemp; stalks and straws; and wood.

There is considerable variation in the amount of cellulose in these different plant tissues. The purest form of native cellulose is the cotton-seed hair, which may contain only 2 per cent non-cellulose material. The long hairs (lint) are used for processing into cotton fabrics while the short hairs (linters) are used for chemical purposes, especially for the manufacture of cellulose esters for plastics and for high-grade regenerated cellulose (viscose). Bast fibres are also used in textiles. Flax and ramie contain 80–90 per cent cellulose whereas jute has only 65–75 per cent. The composition of wood varies with species but generally, on a dry basis, it contains 40–50 per cent cellulose, 20–30 per cent lignin, and 10–30 per cent hemicelluloses and other polysaccharides. Wood is the most widely used of all cellulosic materials. Besides its use as timber and fuel, it is freed from lignin to give wood pulp, the raw material for the production of paper, regenerated cellulose, and cellulose derivatives. Leaf fibres, stalks, and straws all contain large quantities of cellulose but the difficulties of collection and purification limit their industrial utilization. Hemp, cereal straws, bagasse, cornstalks, esparto grass, and many others have, however, been used for the manufacture of cordage, wallboard, and insulating board, and bamboo grass is a source of good-grade paper pulp.

The methods for the isolation and purification of cellulose are dependent on the source of the raw material and the purposes for which it is required. The principle is to subject the native cellulose to reagents which will either dissolve or destroy the non-cellulosic impurities while having as little effect as possible on the cellulose itself. In the case of the cotton fibre, the purification is achieved by mild treatment with dilute alkali, followed by washing and gentle bleaching. The isolation and purification of cellulose from wood requires a more drastic process. This usually involves treatment of wood chips either with a mixture of calcium or sodium bisulphite and sulphurous acid (sulphite process) or with sodium hydroxide alone (soda process) or mixed with sodium sulphide (sulphate process). These are carried out under pressure and at elevated temperatures, and are followed by bleaching treatments. The product, wood pulp, may then be further purified to remove non cellulosic carbohydrates.

Chemical Structure

The evidence obtained by the chemical methods already discussed established that cellulose is made up very largely of D-glucopyranose units linked together through $1\beta \rightarrow 4$ linkages. There wa still a need to show that the β-D-Gp 1—4 D-Gp linkage is uniforn throughout however, since variation in even a small percentage o the sugar units could cause great variations in the physical propertie of cellulose. Freudenberg and his collaborators[69,70] have provide both polarimetric and kinetic evidence to demonstrate that at leas 99 per cent of the glycosidic bonds are β-D-Gp 1—4 D-Gp.

They argued that if the chain were entirely uniform, then it molecular rotation would be an additive function of the rotation of the constituent sugar units. If $(M)_n$ is the molecular rotation o a polymer of n units, $(M)_e$ that of the non-reducing terminal suga unit, $(M)_i$ that of an intermediate sugar unit, and $(M)_a$ that of th reducing terminal unit, then

$$(M)_n = (M)_e + (n-2)(M)_i + (M)_a \qquad \ldots (1$$

Therefore, the rotation per unit is

$$\frac{(M)_n}{n} = \frac{(M)_e + (M)_a}{n} + \frac{n-2}{n}\ (M)_i \qquad \ldots (2$$

Assuming cellulose to be an infinitely long chain of molecula rotation $(M)_\infty$, then the contributions to its rotation by the end units can be neglected. The rotation per unit for cellulose is therefore,

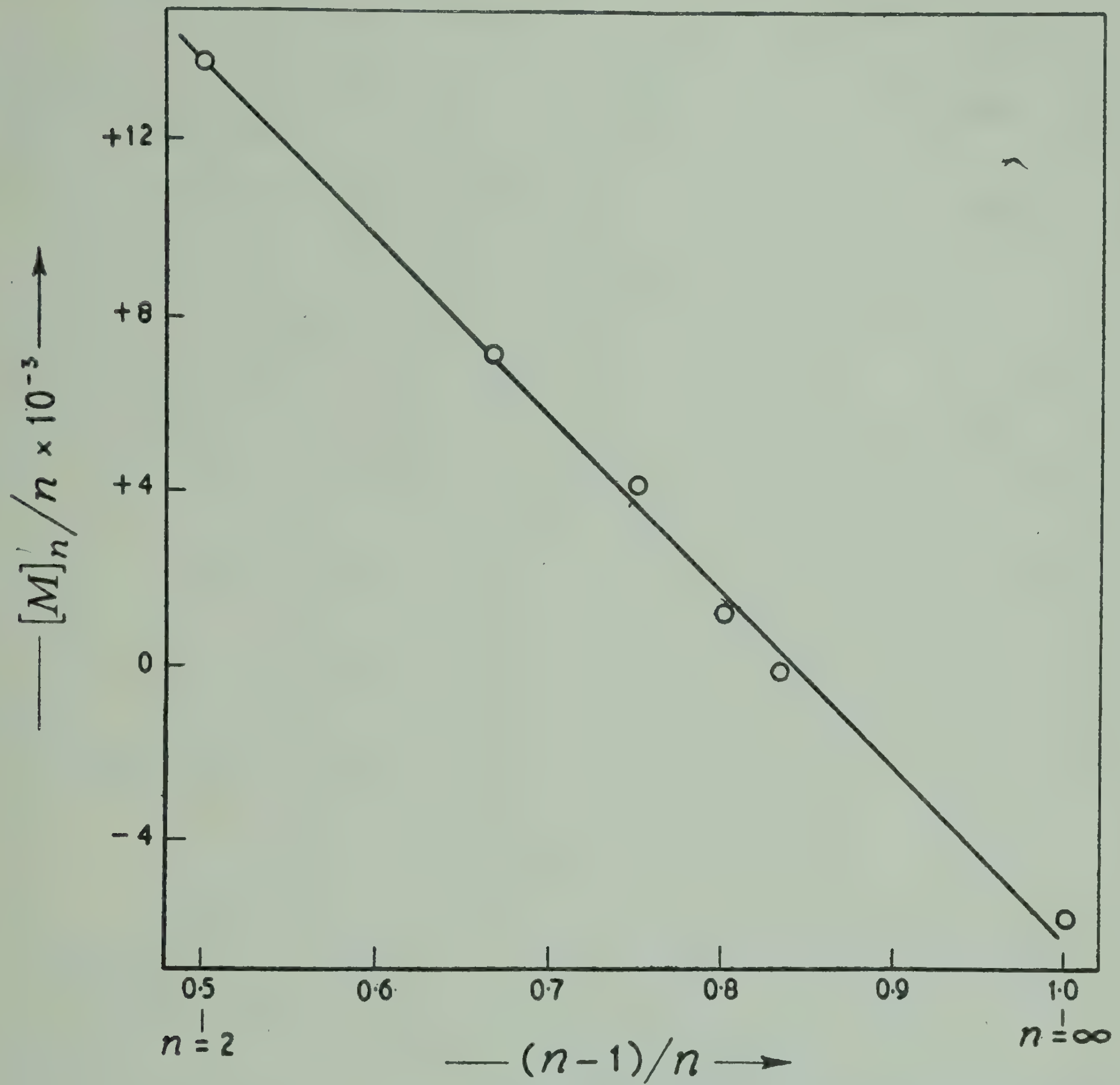
$$\frac{(M)_\infty}{\infty} = (M)_i \qquad \ldots (3$$

Substituting in equation (2)

$$\frac{(M)_n}{n} = \frac{(M)_e + (M)_a}{n} + \frac{n-2}{n}\left(\frac{(M)_\infty}{\infty}\right) \qquad \ldots (4$$

Using these expressions and measuring the molecular rotation of cellobiose, cellotriose, and cellopentaose, it is possible to comput the molecular rotation of cellulose. The calculated value agree very well with that observed, and it was concluded that the cellulos chain is uniform. Alternatively, if the linkage is uniform, th expression $((M)_n/n)$ plotted against $((n-1)/n)$ should give a straigh line for values $n \geqslant 2$ and at $n = \infty$, this line should give the observe value of $((M)_n/n)$ for cellulose. It was found that this linear relation holds, as shown in the graph (see Figure 5), for the series of α-D-acetates of degree of polymerization 1 to 6 inclusively, obtained by acetolysis of cellulose.[26,27]

Kinetic proof was obtained similarly by assuming that all the hydrolysable links in a uniform chain are equal and equivalent except for the terminal units which can be neglected in an infinitely long chain. Cellobiose, cellotriose, and cellotetraose were hydrolysed under standard conditions and the hydrolysis was followed by estimation of the reducing groups produced and also by changes in optical rotation. The observed results agreed excellently with those calculated using formulae based on the above assumption. Also, when cellulose was hydrolysed, the observed results agreed with those calculated, within limits of experimental error, over the whole course of hydrolysis.



(Dickey and Wolfrom,[26] by courtesy of the *Journal of the American Chemical Society*)

Figure 5. Relation between degree of polymerization and molecular rotation

In recent years, a number of workers, using refined techniques and more complex mathematics, have reduced the limit of error in Freudenberg's kinetic proof to about 0·1 per cent of the glucosidic bonds,[71-73] but there still remains the question of whether, in native celluloses, there exist a few weak bonds or cross linkages.[74-80] So far, however, there has been no conclusive evidence to support the suggestion.

Molecular Weight

The average molecular weight of cellulose can be measured by many methods, including the estimation of the reducing and non-reducing end-groups, viscosity and osmotic pressure measurements, ultracentrifugation, light scattering, and interpretations of the streaming birefrigence of cellulose solutions and their behaviour during precipitation with a non-solvent. The chemical methods are handicapped by the need to measure a very low percentage of end-groups and the physical methods have the disadvantage that, in nearly all cases, they must be applied to cellulose solutions. These methods have been surveyed in several reviews.[81-85] Results obtained are highly dependent on the method of preparation of the cellulose samples since severe conditions will cause oxidation and subsequent degradation of the chains by alkali may occur. Using carefully prepared cellulose specimens, however, the physical methods all indicate a value for native cellulose of greater than 3,000 anhydroglucose units (i.e. 1,500 cellobiose units) per chain.

Crystalline Structure

Although the structure of cellulose, as shown opposite, can serve to explain many of its properties, in order to understand its behaviour more fully, it is necessary to consider the spatial arrangement of each atom in each glucopyranose unit and the spatial distribution of these units within the polymer. This is shown diagrammatically in Figure 6. It shows how long threadlike chains are possible.

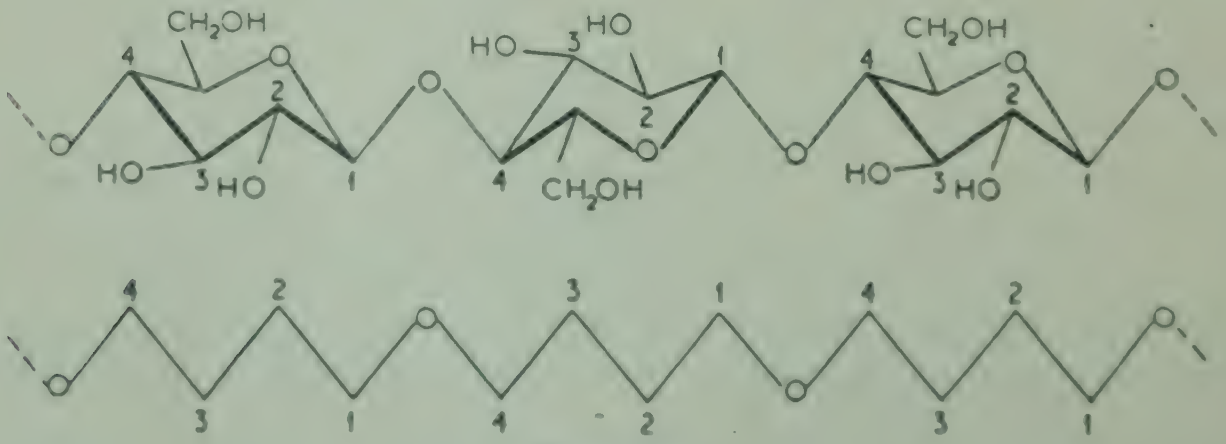
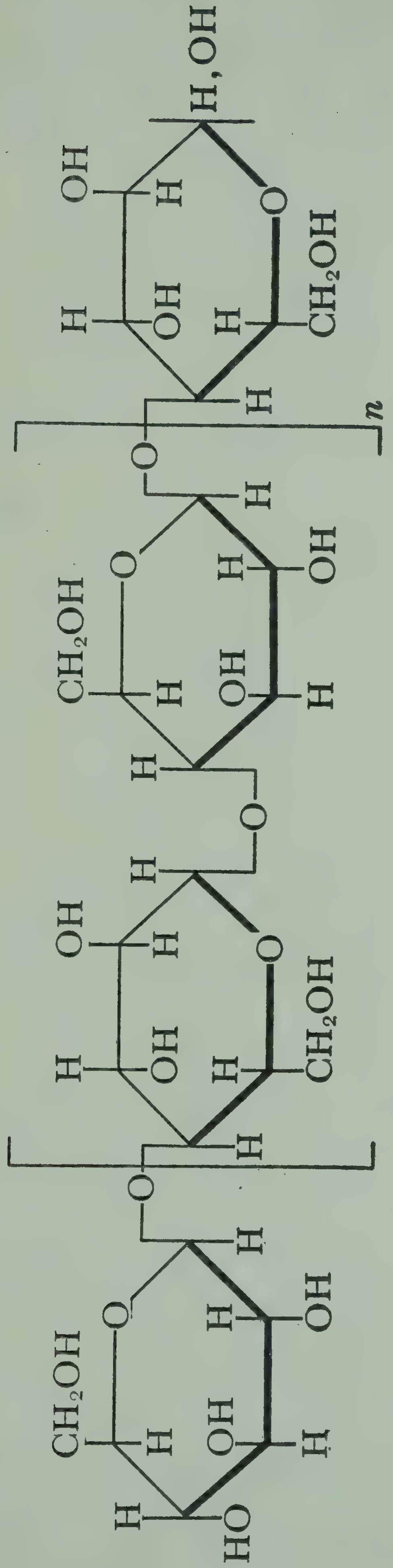


Figure 6.


CH₂OH
H
OH
H
HO
H
OH
H
O
H
O
H
OH
H
CH₂OH
O
H
OH
H
H
O
H
CH₂OH
H
OH
H
OH
H
O
H
O
H
OH
H
CH₂OH
O
H
OH
H,OH
n

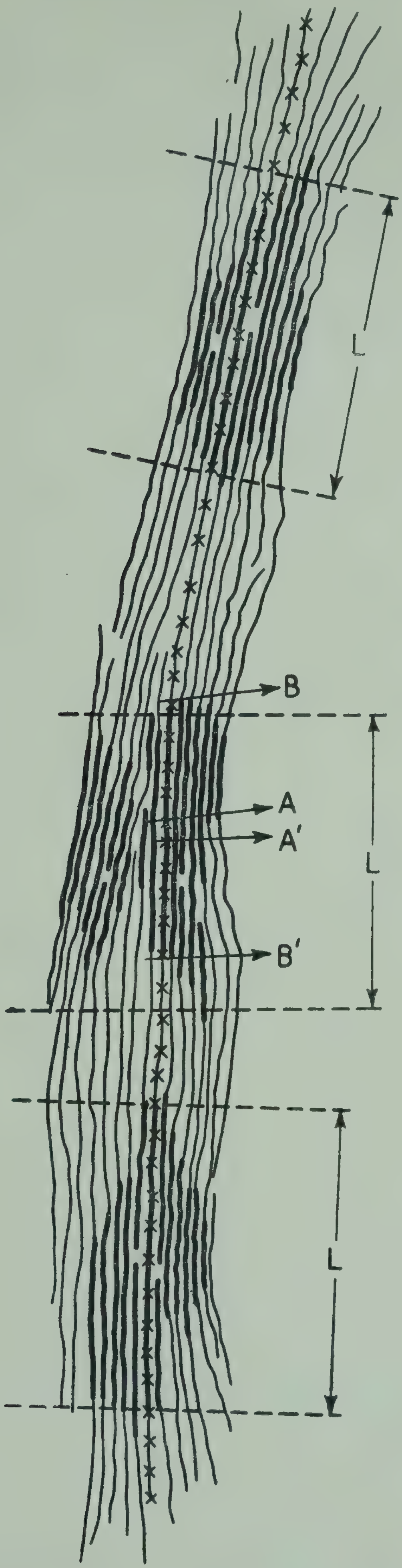
Cellulose

$n \geqslant 1{,}500$

Moreover, the spatial distribution of the hydroxyl groups allows the chains to associate laterally by hydrogen bonding. These intermolecular forces can, in fact, be so regular and highly organized that they confer on the cellulose a three-dimensional regularity characteristic of crystalline materials. This property of cellulose was first detected by X-ray examination, and studies of diffraction patterns has led to the proposal of a unit cell as shown in *The Crystal Structure of Cellulose* (p. 134).[86-88] This unit cell is monoclinic in structure and contains the equivalent of four anhydroglucose residues arranged parallel to the vertical or *b* axis, with cellobiose units at each corner and one through the centre. This means that along the *b* axis the glucose units are firmly held together by β-D-Gp 1—4 D-Gp linkages and the macro-structure of the fibres thus reflects the micro-form of the molecules. Along the *a* axis, the adjacent chains, which point in alternate directions, are held together by hydrogen bonding, while along the *c* axis, the lattice is held by weaker van der Waals' forces. This gives a combination of chain and layer lattices and accounts for many of the properties of cellulose. It must be pointed out that the exact positions of the atoms in crystalline cellulose have not yet been ascertained and the unit cell proposed by Meyer and Misch may be incorrect. This will be dealt with more fully in *The Crystal Structure of Cellulose* (p. 137) and *The Fine Structure of Cellulose* (p. 147).

Cellulose is not crystalline throughout but the crystalline regions are dispersed among smaller regions which do not give well-defined X-ray diffraction patterns. These amorphous regions are made up of chains, which although approximately parallel to the fibre axis, are less highly orientated with a lower degree of hydrogen bonding. A cellulose fibre can be considered as a number of roughly parallel chains which sometimes come together in a highly organized manner to give discrete regions of crystalline nature. A single chain molecule may therefore start in a crystalline region, pass through an amorphous region, and finish up in another crystalline region, and the high strength of the cellulose fibres may be due to the chain ends being held in the lattices by strong hydrogen bonding forces in this manner[89] (see Figure 7).

Generally, the physical properties of cellulose fibres, such as their tenacity, are related to the amounts of crystalline material, while such properties as swelling and ease of chemical reaction are related to the more easily penetrated amorphous regions. The chemical reactivity of the amorphous regions, as opposed to that of the crystalline regions, has, in fact, been used to determine the amounts of each present in cellulose specimens.[88]



(Mark,[89] by courtesy of the *Journal of Physical Chemistry*)

A, A′, B′ are chain-ends within the crystallized region;
B is a chain-end outside the crystallized region;
L is the length of the crystallized region

Figure 7. Main-valence chains going through more than one micella

Although the structure of these chain-bundles determines much of the chemical and physical behaviour of cellulose, its properties are also dependent on the way in which these bundles are orientated with respect to each other, and how they are laid down in the plant cells and fibres. This aspect of cellulose chemistry is still not clearly understood but considerable progress has been made in recent years by use of the electron microscope and ' shadowed ' preparations on the light microscope. The general picture is that the bundles of chains just described associate to form microfibrillae, which in turn group together to give fibrils. The fibrils are laid down at angles to one another, or in spirals of a definite pattern producing layers in the cell walls of wood or in the cellulose fibres of cotton. These layers may alternate with layers of non-cellulosic material such as lignin and pectin, and primary and secondary walls of cellulose are usually distinguishable in the cells or fibres. The nature of the primary wall and the orientation of the fibrils in the secondary wall of cotton play an important role in the characteristics of fibre swelling. The photograph (Figure 8) shows the microfibrillar nature of cellulose.

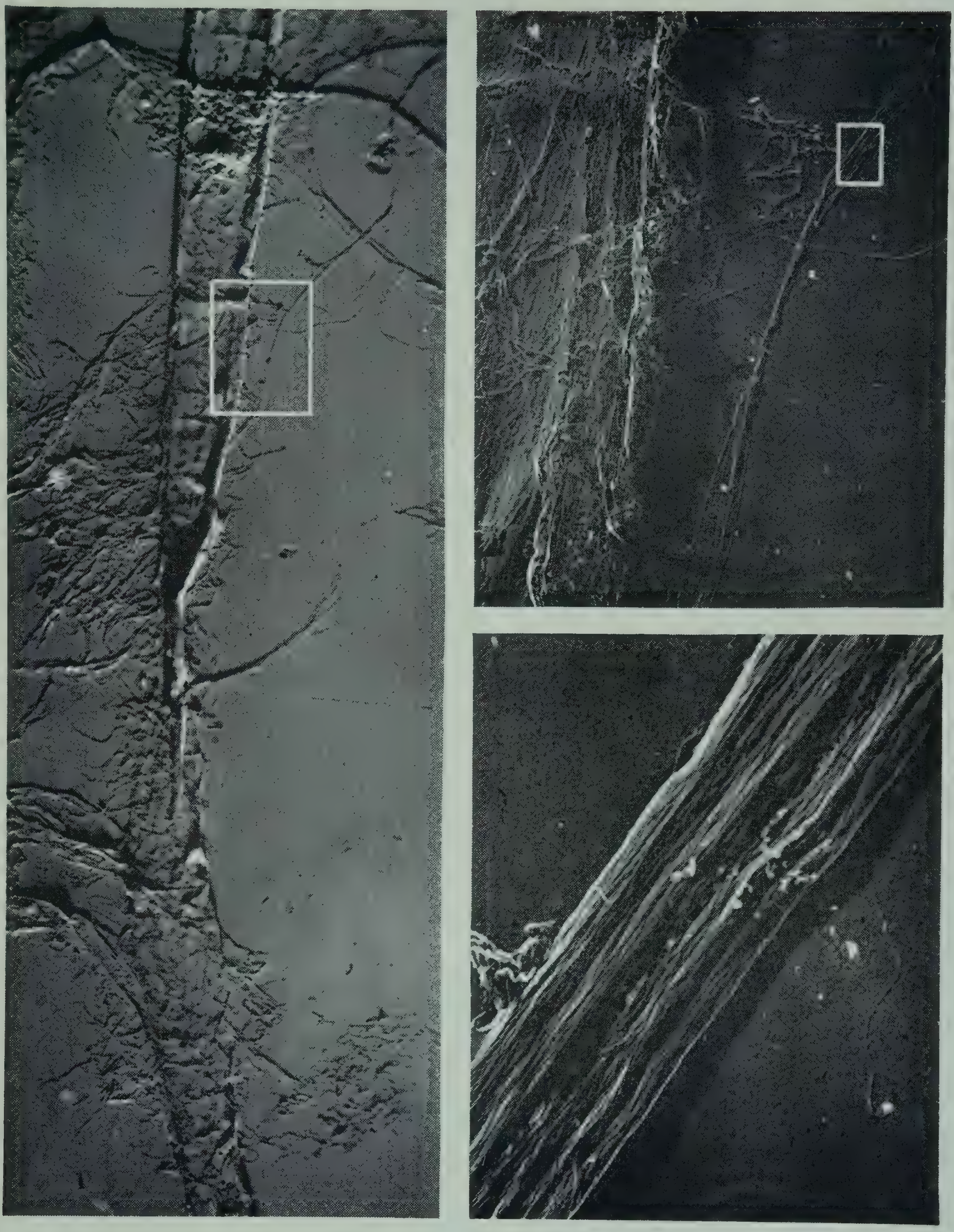
STARCH

Occurrence and Isolation

Starch is the carbohydrate reserve material of higher plants. It may be present in small amounts in green leaves, where it is formed by photosynthetic activity, but the principal storage organs are the seeds, especially of cereals such as wheat, rice, and maize, which may contain up to 70 per cent of starch, and bulbs, tubers, roots, and pithy stems, which contain up to 30 per cent. In order to be transported from the leaves to the storage organs, starch is broken down enzymically to simple sugars which dissolve in the cell sap. These are then reconverted into the polysaccharide for storage. Starch occurs as discrete granules, the shape and size of which are often characteristic of their biological origin. Microscopic identification of many species of starch is thereby possible.[90] The granules are usually made up of layers which partially or completely surround a distinctive marking known as the hilum, and it is thought that these layers are caused by fluctuating conditions of starch deposition, or are the result of physical strains.

The composition of starch is also dependent on its origin. Although mainly composed of polysaccharides of the glucan type, the different starches are also contaminated with varying amounts of fatty acids, protein, and phosphorus.

Starch manufacture is an important industry, and a number of



(Emerson and Page. *J. Roy. Microscopical Soc.* **77,** 113 (1955))

Figure 8. (Left) A light micrograph (× 195) of a metal-shadowed positive replica showing a mechanically treated cellulose fibre over its entire width. (Top right) An electron micrograph (× 875) of a carbon replica of the portion inset of the same fibre. (Bottom right) An electron micrograph (× 9,500) of the portion inset (top right) showing the microfibrillae which are 150-250 Å in width

continuous and batch extraction processes have been developed commercially to separate starch from grains, roots, tubers, and pithy stems. These processes usually involve a series of washing, grinding, and pulping treatments, and fibrous material and germ oil are removed by screening, centrifugation, or rotation operations. In order to obtain starch free from protein, it may be washed out, centrifuged off, or allowed to settle from a slurry. Depending on the source of the raw material, a preliminary steeping process may also be included to facilitate the breakdown of the protein matrix.

Composition

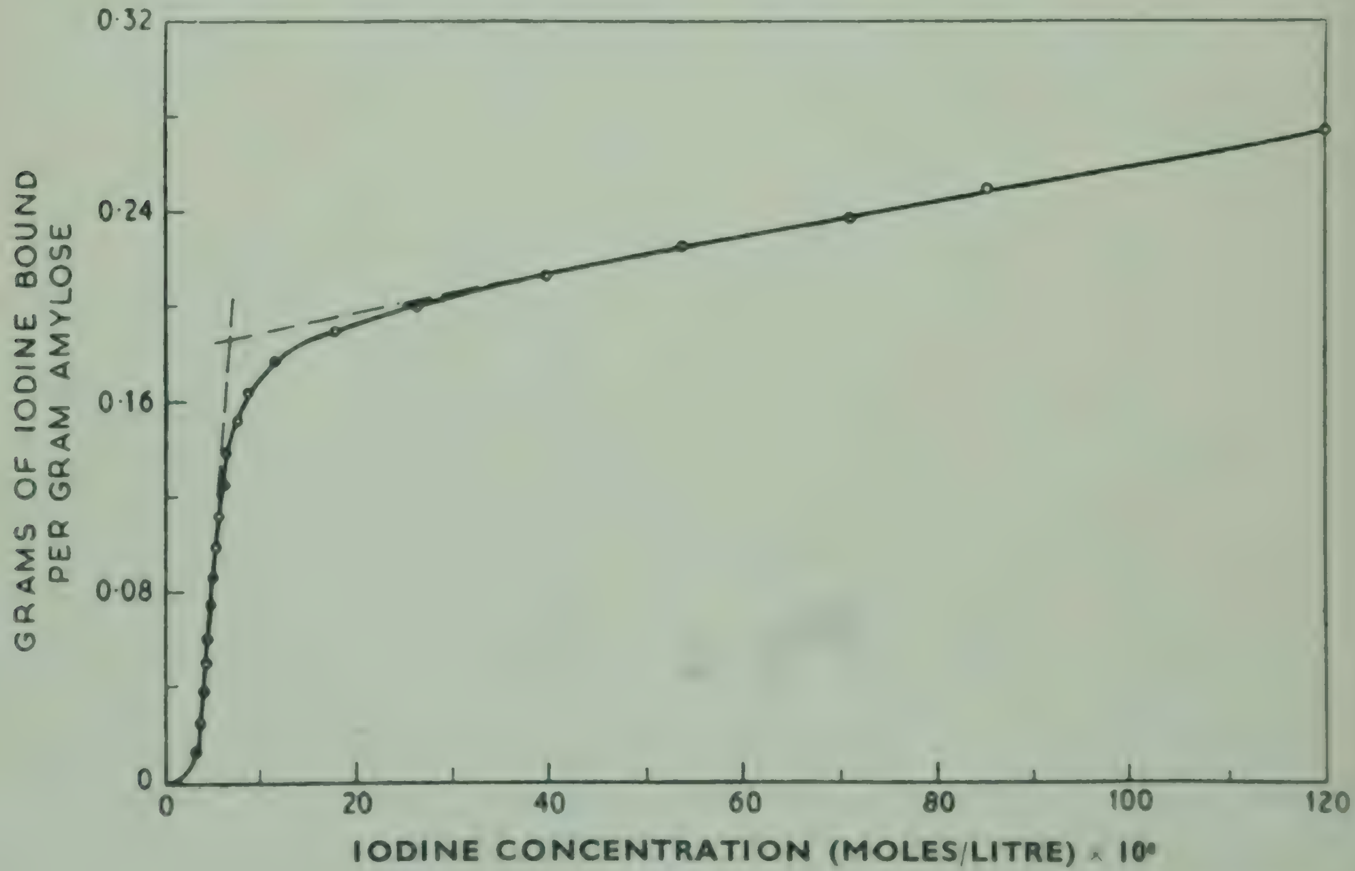
Although starch can be quantitatively converted into D-glucose, it is not homogeneous but a mixture of two structurally different polysaccharides, amylose and amylopectin. Amylose, which is responsible for the blue colour given by starch with iodine, readily undergoes aggregation in solution resulting in deposition of precipitates. This phenomenon is known as retrogradation. Amylopectin, which does not react appreciably with iodine, is responsible for the formation of starch gels. Most starches contain 15–25 per cent amylose (the rest is amylopectin) but there are exceptions, notably those of the waxy or glutinous varieties of maize, rice, and barley, which are almost pure amylopectin. The starches from certain varieties of pea and lily contain up to 75 per cent amylose. The amylose content of various starches is shown in Table 1.

Table 1: Typical starches. Amylose: amylopectin contents

Substance	Amylose (per cent)	No. of glucose units/end group		Calculated length of repeating unit of amylopectin
		By KIO_4	By methylation	
Potato starch	18	28	25	23
Crude potato amylopectin	7	21		20
Sago starch	26	24		18
Banana starch	21	27	24	21
Sweet potato starch ...	18	32	30	27
Arrowroot starch	20	31		25
Tapioca starch	17	25		20
Pearl manioc starch ...	16	24		20
Rice starch	14	24	27	20
Maize starch	23	25		19
Waxy maize starch ...	0	20	20	20
Wheat starch	19	26		21

Fractionation

Many methods have been used to separate amylose and amylopectin, but the most successful depend on the selective precipitation of amylose from starch dispersions. The dispersion may be prepared by heating an aqueous suspension of starch in an autoclave, and a fractionating agent is then added in excess of that required to saturate the water at room temperature. The amylose complexes with the fractionating agent, and on cooling, separates as crystals which are removed. From these, amylose is obtained by adding hot water or ethanol. Amylopectin is then isolated from the mother liquor by precipitation with alcohol or by freeze-drying. The many organic compounds which have been used as fractionating agents include alcohols,[91–94] fatty acids,[95] phenols (including thymol),[96–98] nitroparaffins, nitrobenzene, and amylacetate.[99] Whistler and Hilbert[99] suggest that any water-soluble compound which can form hydrogen bonds with starch is suitable for this purpose. Another method of fractionation involves the preferential absorption of amylopectin on aluminium hydroxide[100] whereas the only commercial process depends on the salting out of amylose from an 8–12 per cent starch suspension by 10–15 per cent aqueous buffered magnesium sulphate.[101]



(Bates, French, and Rundle,[103] by courtesy of the *Journal of the American Chemical Society*)

Figure 9. Amount of iodine bound by 'crystalline amylose' as a function of iodine concentration

In order to test the purity of the fractionation products and also to estimate the ratio of amylose to amylopectin in whole starch, the iodine binding power of amylose is used. When solutions of amylose and amylopectin are titrated potentiometrically with iodine, the amount of iodine bound in complex formation is directly proportional to the amylose content of the solution[102] (see Figure 9).

Amylose

Evidence obtained by chemical methods already described shows that starch is made up of D-glucopyranose units. The α-glucosidic linkage was deduced from the high positive optical rotation of starch and maltose, their hydrolysis by α-glucosidases, and in particular, from Freudenberg's[69,103,104] kinetic studies of chemical hydrolysis. End-group assay by methylation indicated an average chain length of 25 glucose units,[13–16] very much lower than the values obtained by physical methods. After processes had been developed for separating amylose and amylopectin, the methylation work was repeated on the fractions. When amylose was methylated and hydrolysed, the yield of tetra-*O*-methyl-D-glucose corresponded with a chain length of 300–400 units;[16,47,105,106] no significant amount of di-*O*-methyl-D-glucose was isolated. Osmotic pressure measurements on the amylose sample indicated the same chain length, evidence for the unbranched structure of amylose.[105] Determination of the end-group content of amylose by periodate oxidation indicated chain lengths of 250–1,000 units[47,107–109] but, here too, some chain degradation and ' over-oxidation ' can occur.

Determination of the molecular weight of amylose and amylopectin by physical methods, such as ultracentrifugation, light-scattering, osmotic pressure, and viscosity measurements, is more reliable, although difficulties arise because amylose readily retrogrades from solution. Recent results for amylose have indicated a chain length of 3,800 units.[110]

The enzyme, β-amylase, as normally prepared from cereal grains, malt, or soya beans, achieves almost quantitative (greater than 90 per cent) conversion of amylose to maltose.[111] Recently, however, it has been found that crystalline β-amylase from sweet potato[112] converts only 70 per cent of the amylose to maltose, and the presence of a further enzyme, Z-enzyme, is necessary for complete degradation.[113,114] This is ascribed to the presence of anomalous linkages in the amylose, which although preventing the β-amylase activity, can be removed by Z-enzyme. The nature of these linkages is not known with certainty, but it has been suggested that they are β-glucosidic ones.

If the spatial distribution of a chain of glucose units joine 1(α) → 4 is considered, it is found to assume a spiral form as show in Figure 10. This is due to the conformation of the α-glucosidi bonds and is different from cellulose (see Figure 6). Support fo this helical concept has come from X-ray, ultracentrifugal, visco metric, and other studies of the amylose–iodine complex and o certain amylose fractions in solution. Results indicate that th

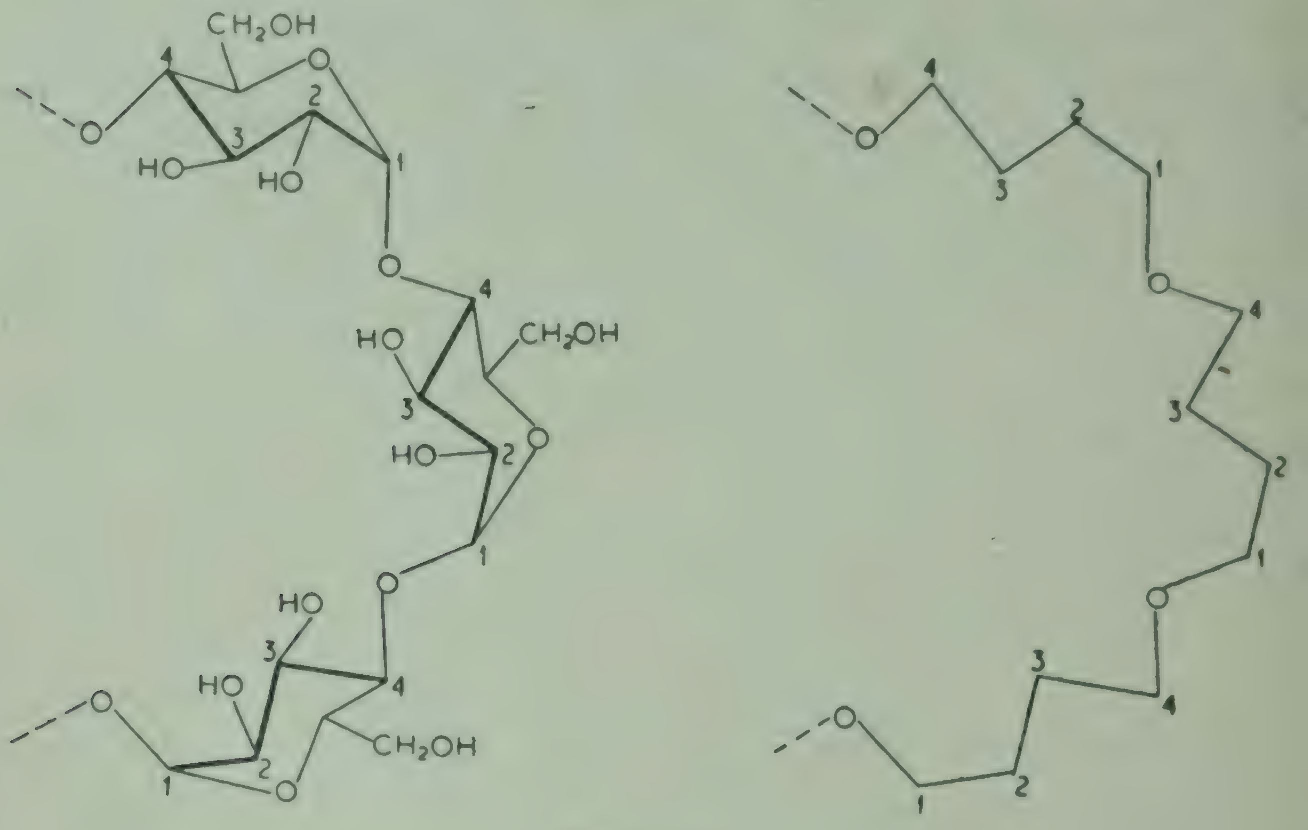
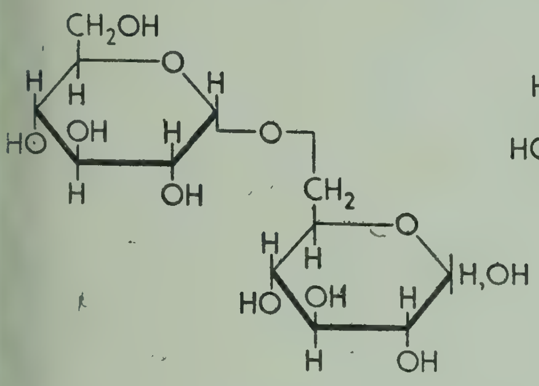


Figure 10.

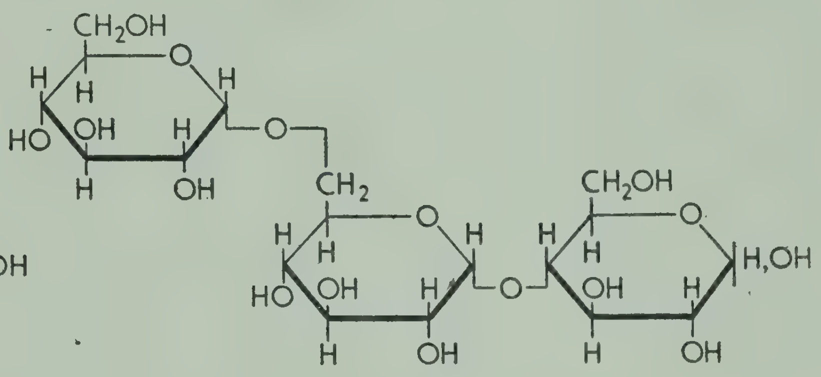
period of each spiral is 6 glucose units and, in the amylose–iodine complex, it has been shown that the iodine atoms are situated in the core of helically orientated amylose molecules.[115–119]

Amylopectin

The methylation[16,47,105,106,108] and periodate[47,107,108] methods show that there are 4–5 per cent of end-groups in amylopectin, corresponding to an unbranched chain of 25–20 D-glucose units. However, amylopectin is almost without reducing power, and molecular weight determinations give values ranging as high as 1,450,000 units.[110] These widely different conclusions can be explained only if amylopectin is highly branched.[120,121] The isolation from the hydrolysate of methylated starch of 2 : 3-di-*O*-methyl-D-glucose and 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose in equimolar amounts, indicates that branching is through α-D-Gp 1—6 D-Gp linkages.[122] There is some uncertainty about the interpretation of these results, however, since there is the possibility of incomplete methylation and also, control experiments have shown that 2 : 3 : 6-tri-*O*-methyl-D-glucose is slightly demethylated under the conditions used during hydrolysis, giving a mixture of di-*O*-methyl-D-glucoses. Periodate oxidation of starch or amylopectin fractions, followed by hydrolysis, yields only 0·5–1·5 per cent of D-glucose, indicating that 75–90 per cent of the branch linkages must be of the 1 → 6 type.[45–48] This follows from the fact that any D-glucose unit joined through $C_{(2)}$ or $C_{(3)}$ as well as $C_{(1)}$ and $C_{(4)}$ will not be oxidized by periodate and so will appear as D-glucose on subsequent hydrolysis. Direct evidence of the existence of 1 → 6 glycosidic linkages in amylopectin has come from the isolation of the crystalline disaccharide, 6-*O*-α-D-glucopyranosyl-D-glucose, known as isomaltose, from waxy maize starch hydrolysed by enzymic[123] or chemical methods.[124]



Isomaltose



Panose

The trisaccharide, *O*-α-D-glucopyranosyl-(1 → 6)-*O*-α-D-glucopyranosyl-(1 → 4)-D-glucopyranose, or panose, has also been isolated from the mixture obtained by the partial hydrolysis of waxy maize starch.[125] The problem arises that isomaltose and panose may not be present in starch but are formed from D-glucose by reversion synthesis, but Wolfrom and his co-workers have shown that the amount of *β*-isomaltose octa-acetate which can be isolated from amylopectin is 200 times as great as the amount formed by acid reversion under similar conditions.[126]

Three types of branched structure for amylopectin have been proposed : Haworth and Hirst's ' laminated ',[127] Staudinger and Husemann's ' herring-bone ' or ' comb ',[128] and Meyer and Bernfeld's ramified or ' tree '.[129] These are shown in Figure 11. The ' laminated ' structure, (*a*), is the simplest and represents an ordered system of single branching which accounts for the observation that the non-reducing end-group values for starch are only slightly altered by large changes in molecular weight. The ' comb ', (*b*), represents one single main chain carrying numerous branches, and was suggested after comparing the viscosities of starch and cellulose derivatives of similar molecular weights. The ramifie structure, (*c*), is the most likely, however, and is supported by th following results of enzymic degradation.[130,131]

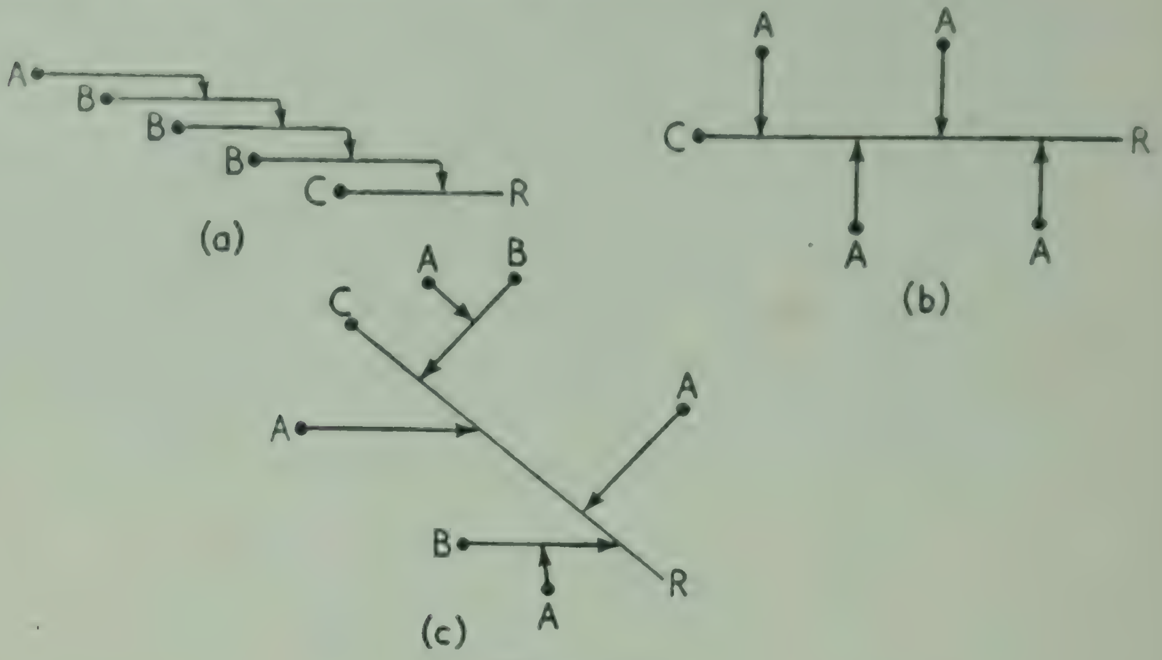


Figure 11.

In Figure 11, side chains linked solely through their reducing group to the rest of the molecule are denoted by A, B chains are those to which A chains are attached but which are themselves similarly linked to other chains, and chains with a free reducing group (R), are denoted by C. In (*a*), where there is only one A chain per molecule of *n* chain units,

$$\frac{\mathrm{A}}{\mathrm{B}} = \frac{1}{(n-2)}$$

in (*b*), where there are no B chains,

$$\frac{\mathrm{A}}{\mathrm{B}} = \infty$$

and in (*c*),

$$\frac{\mathrm{A}}{\mathrm{B}} \sim 1\cdot$$

The action on amylopectin of β-amylase, followed by R-enzyme, has enabled the A : B ratio to be determined. β-Amylase attacks amylopectin, leaving a limit dextrin corresponding to about 50 per cent of the original polysaccharide. This enzyme completely removes the A chains except for ' stubs ' of two or three glucose units, whereas the B chains remain intact, the enzyme action being blocked by the branch points. When this limit dextrin is treated with R-enzyme, $1(\alpha) \rightarrow 6$ linkages are hydrolysed, so the amount of maltose and maltotriose produced is a measure of the number of A chains present. The B chains appear as linear dextrins of $n \geqslant 6$ as a result of the action of R-enzyme. The values of A and A : B obtained experimentally agree with those calculated for a structure of type (*c*) where there is random branching and a submaximal proportion of A chains.

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3

THE HYDROLYTIC AND OXIDATIVE DEGRADATION OF CELLULOSE

INTRODUCTION

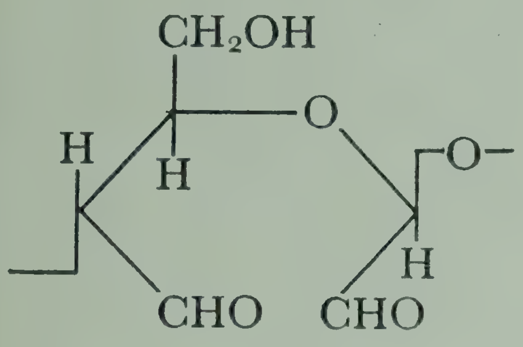
The volume of work on the chemical degradation of cellulose has become so large in recent years that only selected topics are discussed. The selection has of necessity been somewhat arbitrary, depending upon the predilections of the author, and it must not be assumed that work that is omitted is unimportant.

The term ' degradation ' is rather vague in its application to cellulose, and probably has its origin in the deterioration of desirable properties, such as mechanical strength and chemical stability, that may accompany certain common industrial processes. From a chemical point of view, the complete degradation of cellulose is its conversion to carbon dioxide and water. Any intermediate stage in this process may be described as partial degradation. This chapter will deal only with those types of partial degradation that are brought about by acids and by oxidizing agents under such conditions that the main products are water-insoluble solids. These products are called *hydrocelluloses* and *oxycelluloses* respectively, and often differ very little in appearance from the material from which they have been derived. Invariably, however, some loss of strength occurs, and in many cases this loss is sufficient to cause the fibrous material to fall to powder when the degree of chemical modification of the cellulose is quite small. In fact, one of the most striking characteristics of the hydrolytic and oxidative degradation of cellulose is the profound effect on its physical properties of slight changes in its chemical composition.

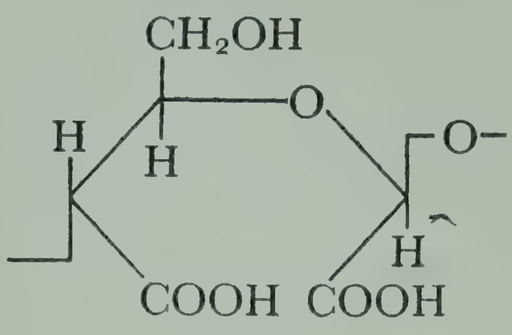
Cellulose may be obtained from a wide variety of plants, as well as to a smaller extent from certain animal sources. For reasons of convenience and of industrial importance by far the greatest volume of work on the degradation of cellulose has been done on ' scoured ' cotton. Scouring consists in boiling the cotton with dilute alkali. For adequate purification for chemical work, it is necessary to boil the cotton under pressure for several hours with 1 per cent sodium hydroxide. To a very close approximation, the resulting material is an unbranched chain of β-D-glucopyranose units linked from $C_{(1)}$ to $C_{(4)}$, of high average chain-length, whose reducing terminal units have been converted into units containing carboxyl groups (see Chapter 4. *The Degradation of Chemically Modified Cellulose by Alkali*). It therefore possesses a very small carboxyl content and virtually no reducing power. Scoured ramie and viscose rayon (itself an oxycellulose) have also been used in studying the degradation of cellulose.

There are numerous ways in which partial degradation of the cellulose molecule may occur. The simplest is by hydrolysis of one or more glycosidic linkages with the production, at each point of scission, of a reducing (hemi-acetal) and a non-reducing end-group. This is what happens in the production of hydrocelluloses by means of dilute acids. Oxidation, on the other hand, can occur either with or without simultaneous chain-scission. The more important types of oxidation that can occur without chain-scission are shown in formulae (I)–(VI). For a particular type of reaction to occur, however, it is necessary for the molecules of the appropriate reagent to be able to reach the functional groups to be attacked. It is a fundamental characteristic of fibrous cellulose that not all the functional groups of a particular sort are equally accessible to a chemical reagent. Thus the hydrolysis of glycosidic linkages by dilute acids proceeds quite rapidly at first, but becomes exceedingly slow after a very small fraction of these linkages have been broken. This heterogeneity in the solid phase is of even greater significance in its effect on the course of the oxidation of cellulose. It may so alter the relative rates of primary and secondary reactions at quite an early stage in the oxidation that their relative importance in determining the nature of the product is completely changed.[1] Hence few oxidants are specific in their mode of oxidizing cellulose, and a knowledge of their reactions with simple alcohols is of little help in predicting how they will react with cellulose. It is therefore customary to designate an oxycellulose by the oxidant used in its preparation.

It is clear from the last paragraph that the supramolecular (or fine) structure of cellulose has an importance equal to its molecular structure in determining the course of hydrolytic and oxidative degradation. For the present purpose it will be sufficient to use the simplified picture of cellulose as a mixture of ' crystalline ' and ' amorphous ' regions. It must be remembered, however, that it *is* a simplified picture, and that there is no sharp line of demarcation between these regions. Rather, there is a heterogeneous structure, of which some parts are more highly ordered than others, and in which a single chain-molecule may pass through several crystalline and amorphous regions along different parts of its length.


CH₂OH
O
H
H
O–
H
CHO
CHO

(I)


CH₂OH
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O–
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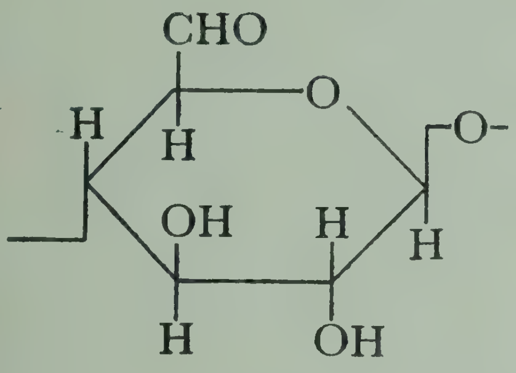
COOH
COOH

(II)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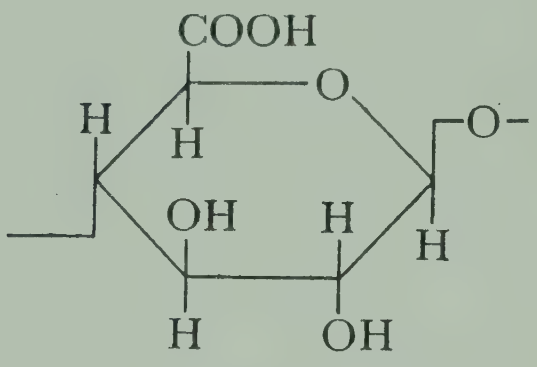
OH

(III)


COOH
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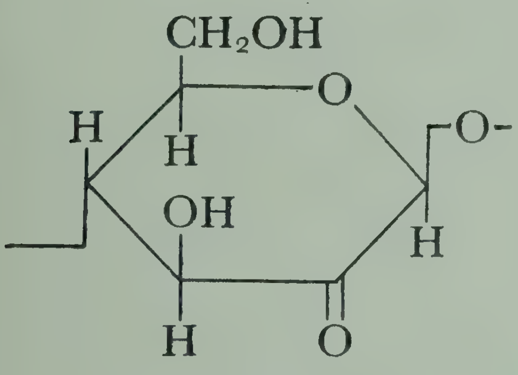
H
OH

(IV)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O–
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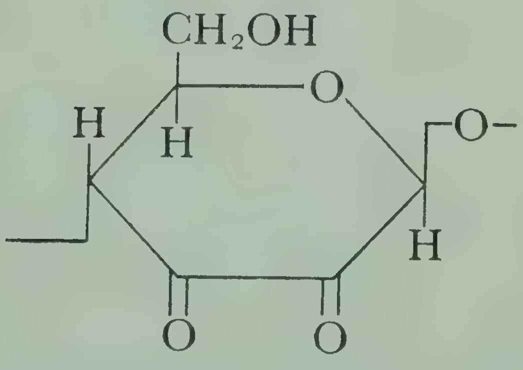
H
O

(V)


CH₂OH
O
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O–
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O
O

(VI)

METHODS OF STUDY

The only method of proving directly the structure of a particular type of degraded cellulose is to hydrolyse it, or a derivative of it, completely, and to isolate from the hydrolysate such monomeric substances as will enable the original structure to be unequivocally deduced. Examples of the application of this method will be given later. It is, however, often difficult to separate very small amounts of a desired substance from the large quantities of glucose arising from the unmodified chain-units in the material. Recent advances in chromatography have provided valuable help in this connection.

Another, less rigorous, but very fruitful, method of investigation was introduced by Clibbens and his collaborators at the Shirley Institute.[1] It consists of preparing series of materials that have been degraded to a progressively increasing extent by means of a particular reagent, and measuring certain properties that are characteristic of the extent of degradation. These properties fall into two groups: those related to average chain-length and chain-length distribution, and those measuring, or related to, functional groups, such as carbonyl and carboxyl groups. The methods of measuring some of these properties will now be considered.

Properties Related to Chain-length

Tensile strength and viscosity in solution are probably the most important properties of hydrocelluloses and oxycelluloses that can be related to average chain-length and chain-length distribution. The great strength of the cellulose fibre is due, firstly, to the strength of the covalent bonds in the individual chain-molecules and, secondly, to the lateral cohesive forces, mainly hydrogen bonds, that prevent adjacent chain-molecules from slipping past one another. Any shortening of the chain-molecules through scission of the glycosidic linkages diminishes the extent of mutual overlapping and hence results in a loss of tensile strength. Ideally, strength measurements should be made on single fibres, but as this is rather tedious, yarn is frequently used. Although in some cases[2] the percentage loss of strength caused by a particular treatment is nearly the same for both yarns and fibres, in others[3] considerable differences are found.

One of the standard methods of determining the average degree of polymerization of a polymer is to measure its intrinsic viscosity in a suitable solvent. For cellulose the most usual solvents are cuprammonium hydroxide and cupri-ethylenediamine, but most of the published formulae for converting the viscosities of solutions of cellulose to average degrees of polymerization are not really satisfactory. Much of the work on the degradation of cellulose has therefore been done with the purely empirical measurement known as the ' cuprammonium fluidity '. This is defined as the fluidity in reciprocal poises of a 0·5 per cent solution of cellulose material in a standard solvent at 20°. It is an inverse measure of chain-length and therefore increases with increasing amounts of chain-scission. It is, however, approximately linearly related to tensile strength, which accounts for its widespread use as a standard test in the cotton industry.

Another empirical measure of chain-length is the so-called ‘nitrocellulose fluidity’ introduced by Davidson.[4] This is the fluidity at 20° of a 0·25 per cent solution in acetone of the cellulose material after it has been fully nitrated under non-degradative conditions. It has proved very valuable in the study of ‘alkali-sensitivity’. Alkali-sensitivity is the phenomenon, exhibited by certain types of oxycellulose, of suffering a great loss of strength on treatment with cold dilute alkali. It is due to the possession by these materials of glycosidic linkages—normally very resistant to alkali—that have been rendered highly susceptible to alkaline scission by reason of the chemical modification undergone by the cellulose. Alkali-sensitivity is strikingly illustrated by comparing the cuprammonium and nitrocellulose fluidities of series of materials. Figure 1 (*a*) shows such a comparison for hydrocelluloses and several types of oxycellulose. If the two sorts of fluidity are both empirical measures of the same thing—average chain-length—there should be

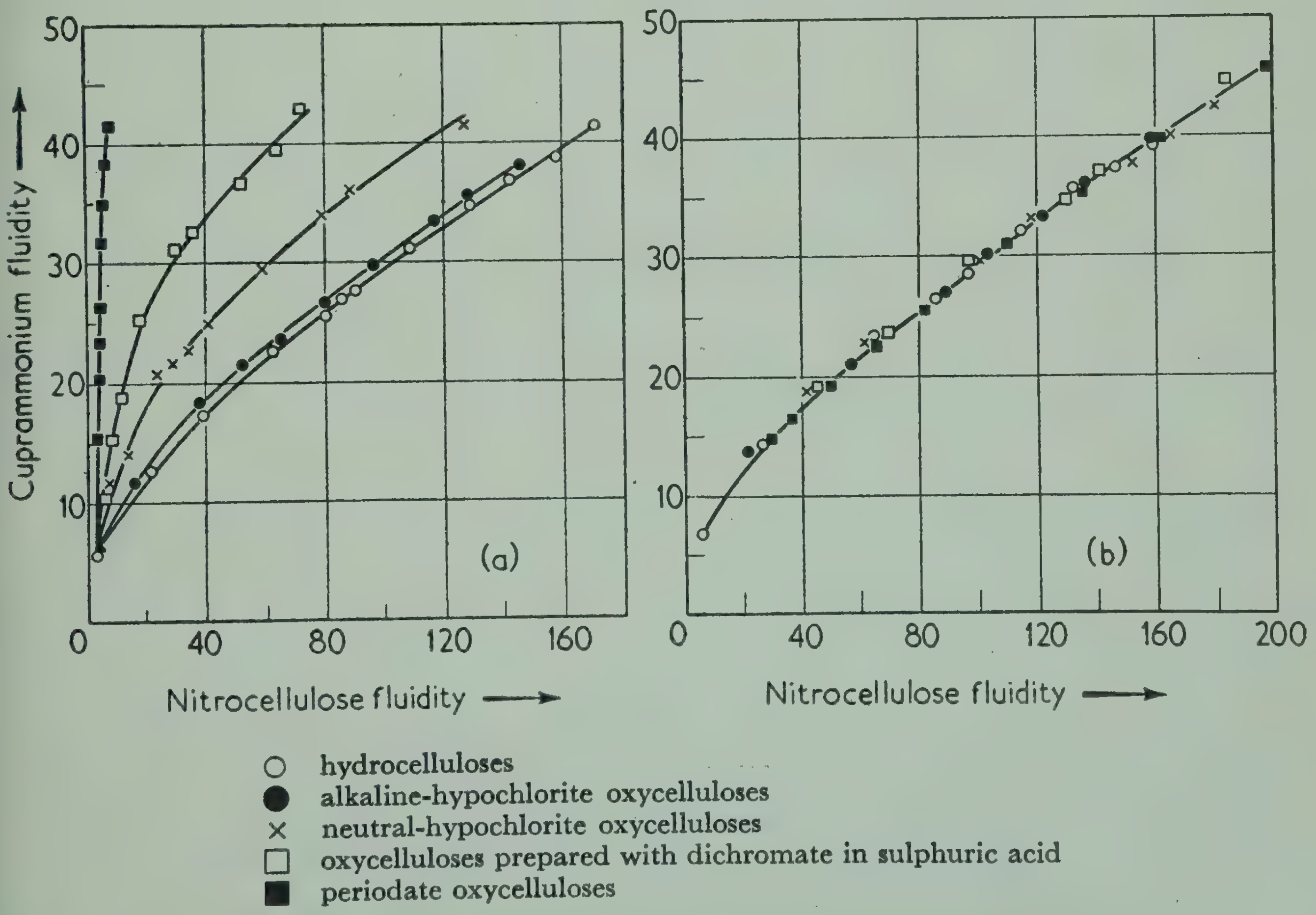


Figure 1. Relation between cuprammonium fluidity and nitrocellulose fluidity for (*a*) *hydrocelluloses and oxycelluloses and* (*b*) *alkali-boiled hydrocelluloses and oxycelluloses*[4,5]

a unique relation between them, whatever the method of degradation of the cellulose. It is apparent that this is not so, however, some materials having a much higher cuprammonium fluidity at a given nitrocellulose fluidity than others. These are the alkali-sensitive materials, whose cuprammonium fluidity is a measure, not of chain-length, but of chain-length after alkaline treatment, cuprammonium hydroxide being a highly alkaline solvent. When the materials whose fluidities are plotted in Figure 1 (*a*) are boiled with dilute alkali before being nitrated and having their cuprammonium fluidities measured the expected unique relation between cuprammonium and nitrocellulose fluidity is obtained (Figure 1 (*b*)) and, moreover, the relation is identical with that shown in Figure 1 (*a*) for hydrocelluloses that have not been alkali-boiled. Thus hydrocelluloses are not alkali-sensitive in the sense in which the term is used here.

Determination of Functional Groups

The most important functional groups that can be introduced into the cellulose molecule during degradation are aldehyde groups, potential aldehyde groups (as in hemi-acetals), ketone groups, and carboxyl groups. The first three may be grouped together under the general heading of carbonyl groups. No satisfactory general method of determining such groups exists, although certain methods give reliable results with particular types of material. All claims to have measured either aldehyde or total carbonyl contents of cellulose materials should therefore be examined critically. On the other hand, methods of determining carboxyl groups are established on a much firmer basis and can, as a rule, be relied upon. A selection of methods in common use for determining carbonyl and carboxyl will now be discussed.

Copper number. The time-honoured property of a cellulose material known as the copper number is an empirical measure of reducing power towards hot alkaline copper solutions. It is defined as the amount of copper reduced from the cupric to the cuprous state by 100 g of dry material under certain specified conditions. It bears no known relation to the number of reducing groups present. There is, in fact, good reason to believe that the actual position of an aldehyde group in a modified-cellulose molecule affects its reducing power considerably.[6] Aldehyde groups are the main cause of copper-reducing power in degraded celluloses but it is probable, though not certain, that ketone groups also confer this property.

In spite of the obvious defects of this measurement it has proved of great value in cellulose research for comparing the degrees of degradation of the members of a series of hydrocelluloses or oxycelluloses, and for diagnosing the presence or absence of reducing groups in a material. Scoured cotton, which possesses no reducing groups, has a copper number of zero.

Determination of aldehyde groups. The two methods most frequently used for the determination of aldehyde groups both depend on the oxidation of these groups to carboxyl groups, the oxidants being chlorous acid and alkaline solutions of iodine respectively.

It has been found that when a periodate oxycellulose is treated with a 0·2M solution of sodium chlorite acidified to pH 3 with acetic acid at 20° its carboxyl content rises fairly rapidly to a constant value.[6] (For materials containing more than 150 millimoles of aldehyde groups per 100 g, more concentrated solutions of sodium chlorite are required.) The maximum yield of carboxyl groups varies from 74 per cent to 90 per cent (according to the degree of oxidation of the material) of that calculated on the assumption that the consumption of one molecule of periodate in the preparation of the material results in the production of two aldehyde groups, which are subsequently converted into two carboxyl groups by the chlorous acid. As the carboxyl content rises the copper number falls progressively to nearly zero, thus showing that all the aldehyde groups are accessible to the chlorous acid. Possible explanations of why a 100 per cent yield of carboxyl groups cannot be obtained will be given later (p. 96). Meanwhile, determination of the increase in carboxyl content on treatment with chlorous acid may be regarded as a reasonably satisfactory method of measuring the aldehyde content of a periodate oxycellulose. Application of the method to hydrocelluloses is far less satisfactory, however. Treatment of these materials with chlorous acid does not result in their attaining constant carboxyl contents or in the virtual elimination of copper reducing power within a reasonable length of time.[7] This is illustrated in Figure 2 in which copper number is plotted against carboxyl content for various treatments of three hydrocelluloses. The lowest copper numbers plotted are the lowest that could be obtained without the production of carboxyl groups by reactions other than the oxidation of the aldehyde groups originally present in the hydrocelluloses. The only method of determining the maximum increase in carboxyl content due to these groups is therefore to extrapolate the curves relating copper number and carboxyl content to zero copper number, as

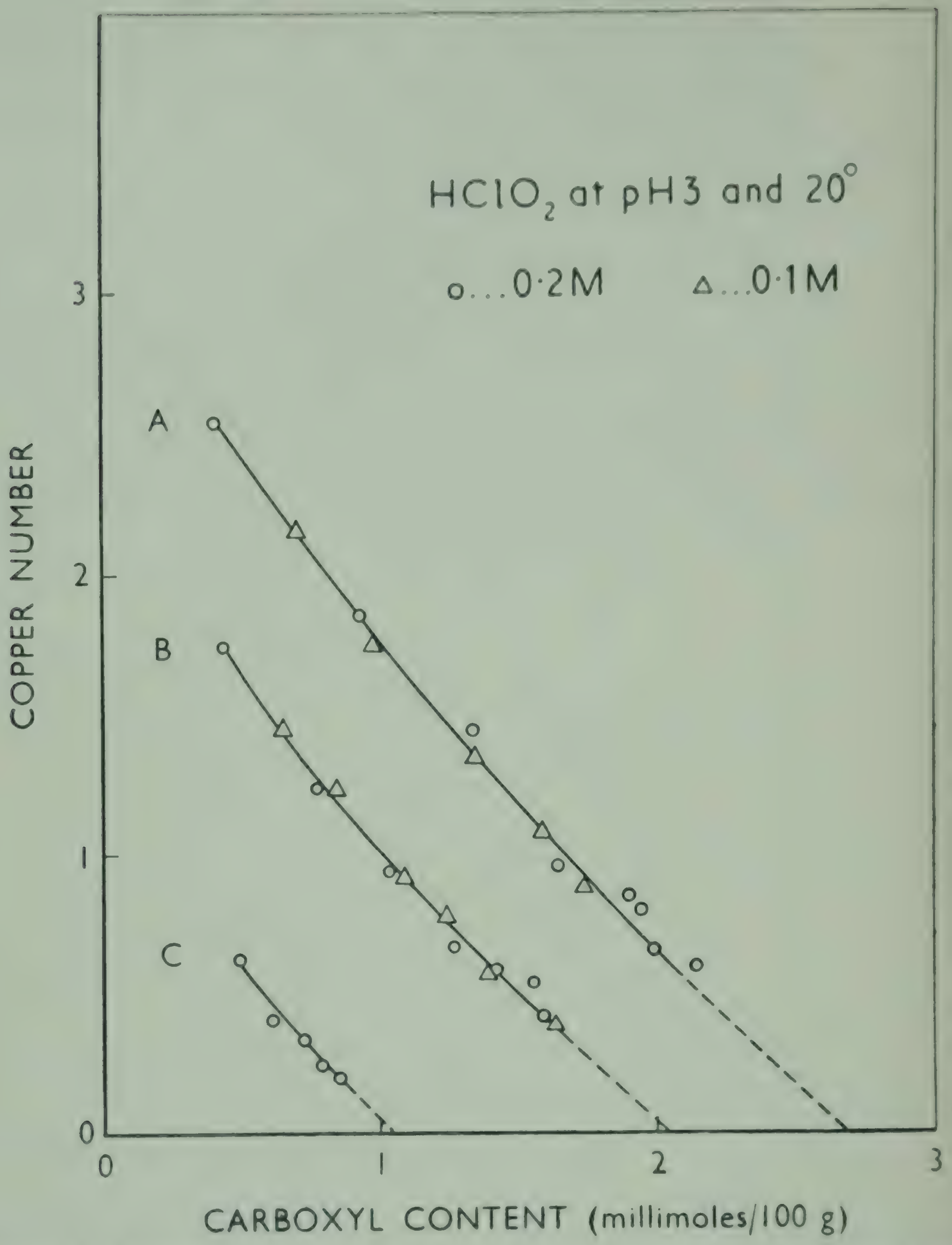


Figure 2. Relation between copper number and carboxyl content for three hydrocelluloses treated with chlorous acid[7]

shown by the broken lines in Figure 2. Such an extrapolation is inevitably rather arbitrary.

The so-called hypoiodite method for measuring aldehyde groups has been subjected to much criticism, but it has continued to be used, notably by Kaverzneva and her collaborators[8] in their attempts to elucidate the mechanism of the oxidation of cellulose by various reagents. The 'hypoiodite' consists of an alkaline solution of iodine, and it is probable that the oxidizing species is undissociated hypoiodous acid.[9] This is present even in alkaline solution owing to its very small dissociation constant. The reactions involved may be represented by equations (1) and (2)

$$I_2 + OH^- \rightleftarrows HIO + I^- \qquad \ldots (1)$$

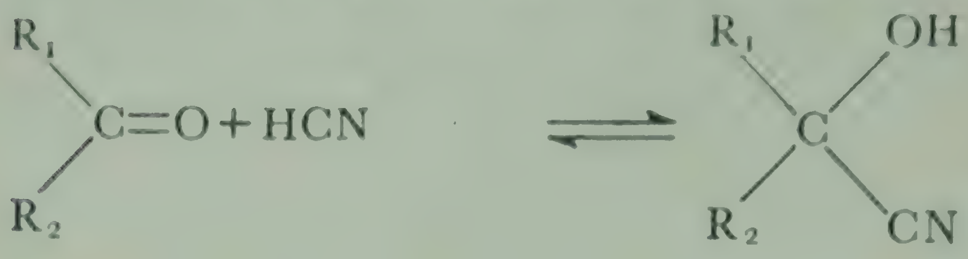
$$R{\cdot}CHO + HIO \rightarrow R{\cdot}COOH + HI \qquad \ldots (2)$$

The amount of iodine converted to iodide is determined by thiosulphate titration of the acidified solution and is used to calculate the number of aldehyde groups oxidized. Conditions must be chosen so that disproportionation of the hypoiodite, which occurs according to equation (3),

$$3IO^- \rightarrow IO_3^- + 2I^- \qquad \ldots (3)$$

does not proceed so rapidly that the solution loses its oxidizing power towards aldehyde groups before their conversion into carboxyl groups is complete. This disproportionation is not detectable by thiosulphate titration since three molecules of hypoiodite liberate the same amount of iodine as one molecule of iodate on reacting with iodide ions in acid solution. It has been found that, from this point of view, 0·01M iodine at pH 10·8 and 0° is suitable for use with hydrocelluloses.[10,11] However, scoured cotton, which contains no aldehyde groups, consumes iodine from this solution in an unknown way, so that the aldehyde content of a hydrocellulose is in fact represented by the difference between its iodine consumption and that of unmodified cotton at a time when both materials are consuming iodine at the same rate. For materials of low aldehyde content this difference is equivalent to a very small thiosulphate titre difference and is difficult to reproduce at all accurately. The main objection to the method, however, is the same as that to the chlorous acid method : the copper number of a hydrocellulose cannot be reduced to nearly zero by means of the alkaline iodine solution. The method is even more unsuitable for use with alkali-sensitive oxycelluloses since the chain-scission resulting from the alkalinity of the reagent produces new reducing groups while the measurement is being made. Reduction of the pH to 9·2 does not overcome this difficulty.[11]

Determination of aldehyde and ketone groups together. Most of the methods of measuring the total content of carbonyl groups in a cellulose material employ one of the well-known addition or condensation reactions of these groups. Typical reactions are those with hydrogen cyanide, phenylhydrazine, and hydroxylamine, represented by equations (4), (5), and (6), respectively



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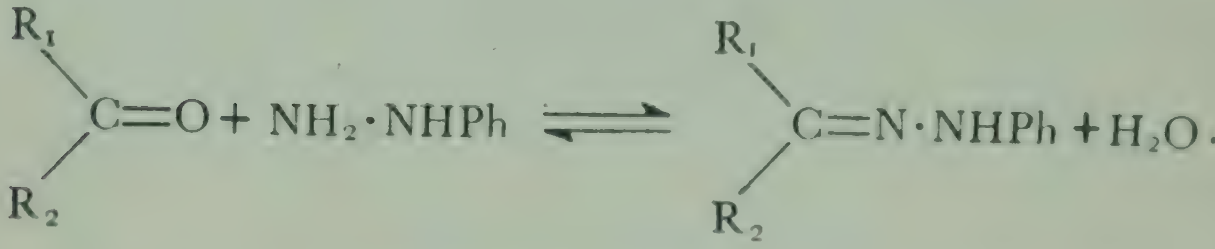

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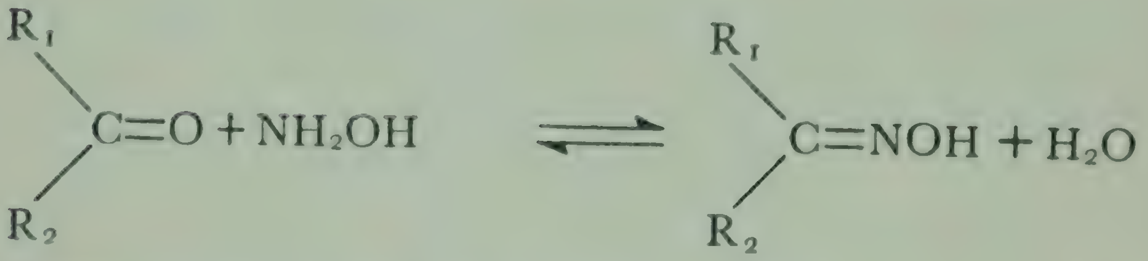


. . . (6)

These reactions are all reversible and the extent of addition or condensation at equilibrium is dependent upon pH; so also is the rate of attainment of equilibrium.[12] The choice of the correct pH is therefore very important if any of these reactions is to be used quantitatively. Unfortunately, as will be shown, this seriously limits the application of the methods for other reasons.

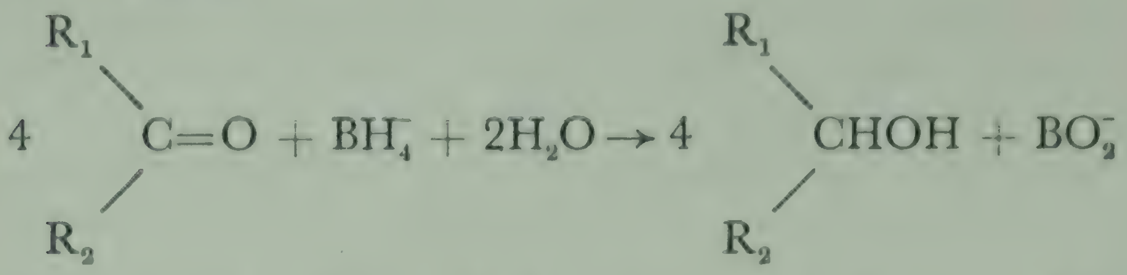
The formation of a cyanohydrin requires a pH of 9 or 10 and is therefore unsatisfactory for use with alkali-sensitive materials. It has been applied to hydrocelluloses,[13] and Isbell[14] has suggested that the use of ^{14}C- labelled cyanide would facilitate the estimation of the amount of cyanohydrin formed.

Phenylhydrazine itself has not proved a very satisfactory reagent for carbonyl-group determinations, but Müller,[15] and Geiger and Wissler,[16] have proposed some more promising methods involving the use of substituted bases such as phenylhydrazine-*p*-sulphonic acid, the amount taken up being estimated by coupling the product with a diazonium salt and measuring the colour developed with a reflectance colorimeter. These methods have been used extensively by Pinte and Rochas,[17] though generally in a qualitative manner; precise results could hardly be expected from reflectance measurements since they must be considerably affected by the nature of the surface of the sample. It has also been suggested[15] that the amount of phenylhydrazine-*p*-sulphonic acid taken up may be estimated by neutralizing the sulphonic acid group with methylene blue. This might well be developed into a satisfactory procedure, since the methylene blue absorption of the phenylhydrazine could be determined from the fall in concentration of the solution of the dye.

The most widely used reagent for determining carbonyl groups in degraded celluloses is hydroxylamine.[18] The usual procedure is to treat the material with a solution of hydroxylamine hydrochloride under such conditions that the final pH is between 3·5 and 5. The amount of hydroxylamine consumed in oxime formation is then determined by titrating the hydrochloric acid liberated. The system is highly buffered and a precise end-point is difficult to obtain, either potentiometrically or by means of an indicator. Thus only a low degree of accuracy can be expected with materials of low aldehyde content. A further factor tending to impair the overall accuracy of the method is the instability of hydroxylamine in solution. Proposals for overcoming this difficulty include the rigid exclusion of oxygen from the reaction system,[19] and the use of the more stable *O*-methylhydroxylamine in place of hydroxylamine itself.[20]

There are two further objections to all methods of carbonyl-group determination employing organic bases. The first is that, being bases, these substances neutralize an unknown proportion of any carboxyl groups that may also be present in the samples. This could only be avoided by carrying out the condensations at pH's either sufficiently high to render neutralization of carboxyl groups virtually complete or sufficiently low to render it negligible. Such pH's are not suitable for the condensation reactions. The suggestion that prior neutralization of the carboxyl groups with calcium or sodium ions effectively protects them from combining with any of the organic base is erroneous because neutralization is essentially a process of ion exchange and all cations present are in competition for the carboxyl groups. It is claimed that the procedure works in practice, however.[18,20] The second objection is that there is, as yet, no means of knowing whether all the carbonyl groups in a material are accessible to the condensing reagent. This information is not easy to obtain and probably the best that can be done is to find several different methods that give the same result with the same material. Work on the comparison of various methods of carbonyl-group determination has been started by Ströle.[19] He has found very good agreement between the hydroxylamine method and the entirely independent borohydride method with periodate oxycelluloses.

The borohydride method, introduced by Lindberg and Theander,[21] consists in reducing the carbonyl groups to alcohol groups by means of an alkaline solution of sodium or potassium borohydride

 . . . (7

$$4\ \mathrm{R_1R_2C{=}O + BH_4^- + 2H_2O \rightarrow 4\ R_1R_2CHOH + BO_2^-}$$

The amount of borohydride consumed is determined by measuring the difference between the volumes of hydrogen produced from the test solution and a ‘ blank ’ solution, on acidification

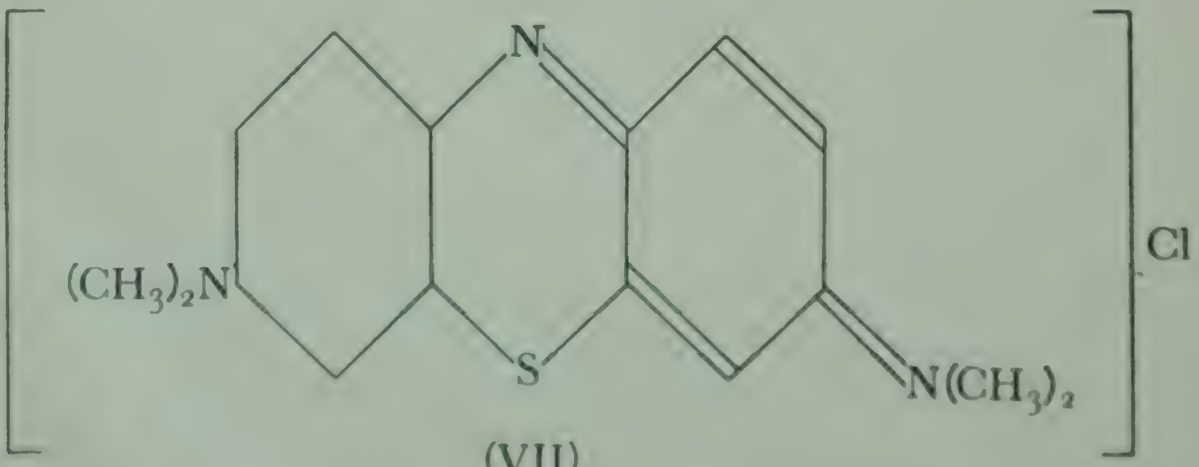
$$\mathrm{BH_4^- + H^+ + 2H_2O \rightarrow HBO_2 + 4H_2} \qquad . . . (8)$$

It is essential that the ‘ blank ’ solution be treated in exactly the same way as the test solution because, even under alkaline conditions, borohydride is not completely stable. With periodate oxycelluloses that had never been dried, Lindberg and Theander obtained results in good agreement with the aldehyde contents calculated from the amounts of periodate reduced during the preparation of the materials. It is clear that the method is unsuitable for hydrocelluloses, however, because the excess of borohydride required for their complete reduction is so large[22] that the difference between the volumes of hydrogen from the test solution and the blank solution cannot be measured with any degree of accuracy.

Determination of carboxyl groups. Nearly all the methods of determining carboxyl groups in cellulose materials are based on cation exchange reactions of the type shown in equation (9)

$$\mathrm{R{\cdot}COOH + M^+ \rightleftarrows R{\cdot}COOM + H^+} \qquad . . . (9)$$

Various sources of cations have been recommended, calcium acetate,[23] silver nitrophenate,[24,25] sodium bicarbonate,[26] and methylene blue chloride (VII),[27]



(VII)

being among the more important. In order to ensure that the exchange goes substantially to completion it is necessary that the concentration of hydrogen ions at equilibrium shall be reasonably low. This is achieved, with the first three reagents mentioned above, by using a large excess of the salt of a weak acid. In spite of somewhat unfavourable conditions, the amount of hydrogen ion liberated, or the amount of cation exchanged, can be determined quite satisfactorily by titration. It is essential, of course, for the cellulose material to be in its free-acid form before being analysed. Acid-washed material should therefore always be used.

Methylene blue chloride differs from the other salts recommended in being derived from both a strong base and a strong acid. A high final pH can therefore only be obtained by the addition of buffer salts. Although this entails the presence of other cations, usually sodium ions, that compete with methylene blue ions for the carboxyl groups, the ‘ affinity ’ of the methylene blue ions for these groups is so much greater than that of the other ions that the resulting error is negligible. The variation of the methylene blue absorption of an oxycellulose with pH is illustrated in Figure 3. Figure 4 shows the effect of the ratio of the concentrations of sodium and dye cations at equilibrium on the methylene blue absorption of four different types of oxycellulose. It is clear from these figures that a reasonably small value of this ratio (the value 4 was chosen by Davidson) and a pH in the neighbourhood of 8 are sufficient to ensure virtually complete

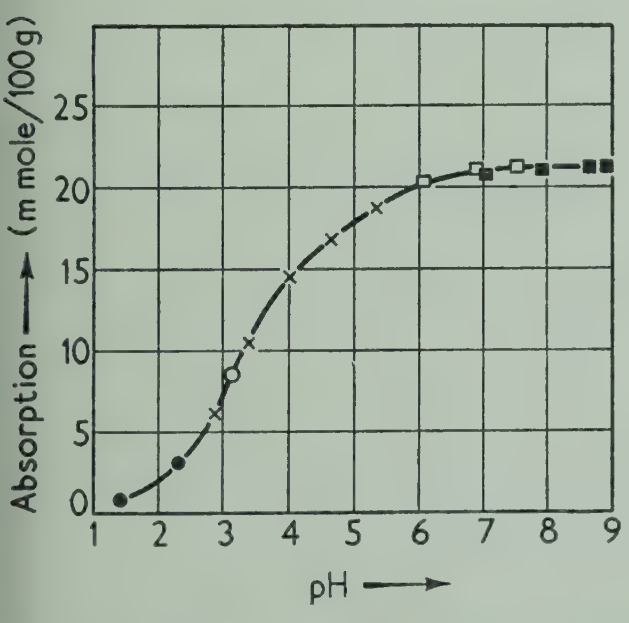


Figure 3. Relation between methylene blue absorption of an acidic oxycellulose and the pH at equilibrium[27]

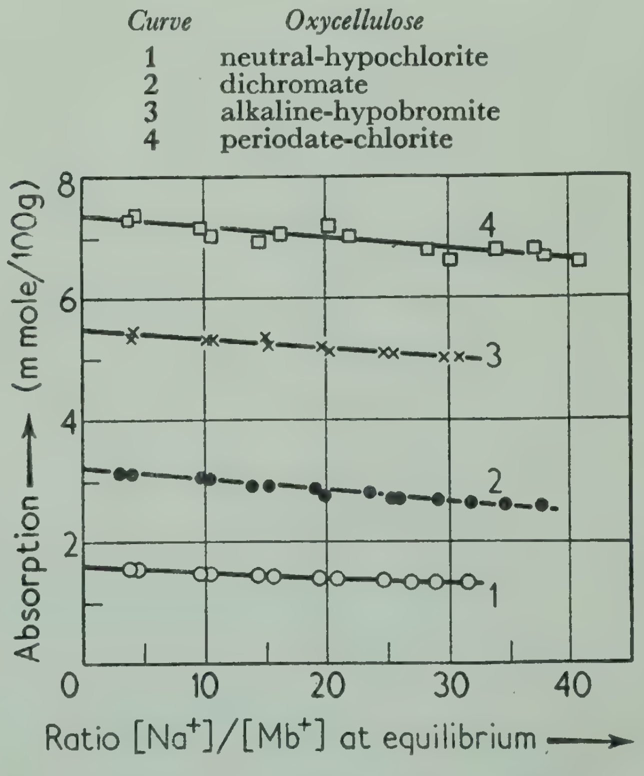


Figure 4. Relation between methylene blue absorption of acidic oxycelluloses and the ratio $[Na^+]/[Mb^+]$ *at equilibrium*[27]

neutralization of carboxyl groups by methylene blue. The measurement of methylene blue absorption has two advantages over othe ion-exchange methods of determining carboxyl groups. First, th experimental technique, namely colorimetric determination of th fall in concentration of the dye solution, enables very small carboxy contents to be measured with a much higher degree of accuracy tha is possible with any titrimetric method. Secondly, owing to th high relative ' affinity ' of methylene blue cations for cellulosi carboxyl groups, it is unnecessary for the material to be converte into its free-acid form before use.

The method of Neale and Stringfellow[28] for determining carboxyl groups is also essentially an ion-exchange method. It consists in treating the cellulose material with dilute sodium hydroxide con-

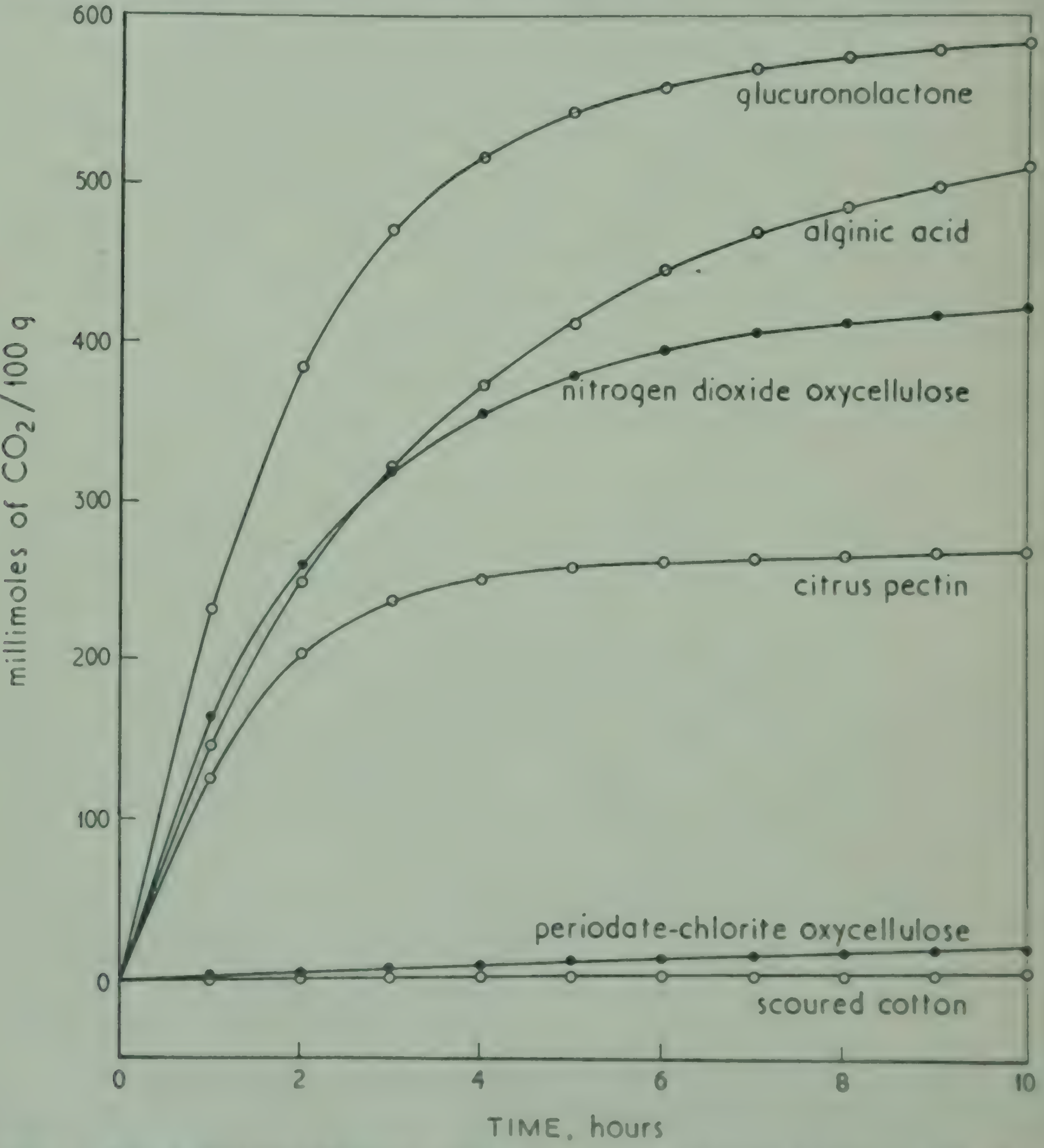


Figure 5. Rate of evolution of carbon dioxide from various substances on boiling with 12 pe cent hydrochloric acid[30,50]

taining a large amount of sodium chloride, and titrating the excess with hydrochloric acid to the bromocresol-purple end-point in the presence of the cellulose. Although the method works well in certain cases it is valueless for materials containing reducing groups, since these are converted into acidic groups by the treatment with sodium hydroxide.[29]

Carboxyl groups that have been formed by oxidation of the primary alcohol groups in cellulose, known as uronic acid groups, can be determined roughly by the method of Lefèvre and Tollens. This consists in measuring the amount of carbon dioxide evolved when the material is boiled with 12 per cent hydrochloric acid. The rate of evolution of carbon dioxide varies from one substance to another.[30] This is illustrated in Figure 5, which shows that whereas citrus pectin is completely decarboxylated in about 5 hr, alginic acid continues to lose carbon dioxide even after 10 hr. Glucuronolactone and a nitrogen dioxide oxycellulose containing a large number of glucuronic acid chain-units appear to be completely decarboxylated in 10 hr, which is the time recommended when the method is applied to oxycelluloses.[30] The specificity of the method for uronic acid groups is demonstrated by the curve for a periodate–chlorite oxycellulose. This material, containing 200 millimoles of non-uronic carboxyl groups per 100 g, yields very little carbon dioxide when boiled with 12 per cent hydrochloric acid. Scoured cotton yields even less.

HYDROCELLULOSES

It is generally accepted that the formation of hydrocelluloses by the action of acids on cellulose consists solely in the hydrolysis of glycosidic linkages. This accords with the fact that the copper number, cuprammonium fluidity, and tensile strength of a hydrocellulose are uniquely related to one another, irrespective of the conditions under which the hydrocellulose has been made.[2] These three properties are all functions of the chain-length distribution in materials that differ only in the number of glycosidic linkages that have been broken; they must, therefore, be related to one another. A direct proof that the acid hydrolysis of cellulose leads to end-groups that can be oxidized to gluconic acid end-groups has been obtained by Blair and Reeves.[31] They prepared a highly degraded hydrocellulose, oxidized it with iodine at pH 10·6, and subjected the product to methanolysis. From the solution they obtained a reasonably good yield of D-gluconic acid, which they identified as its phenylhydrazide, its amide, and its methylamide.

There has been much discussion in recent years of the possibility of the presence of certain linkages in cellulose that are more sensiti to acid hydrolysis than normal β-D-glucosidic linkages. Suc linkages have been described as ' acid-sensitive '. A recent study of the kinetics of the acid degradation of cellulose shows th unmodified cotton contains very few, if any, acid-sensitive linkage but that such linkages may be readily introduced by small amoun of oxidation under alkaline conditions.

The loss of weight during the acid hydrolysis of a sample c cellulose has been made the basis of one method of estimating i content of amorphous material. A review indicating, among othe things, the relation between this and other methods has bee published by Ward.[33] The weight loss is not necessarily directl related to the number of bonds broken, however, and Sharples[3 has proposed a method of determining the fraction of bonds availabl for hydrolysis from a study of the rate of decrease of chain-lengt during acid treatment. By comparing the rates of hydrolysis c cellobiose, unmodified cotton, and a highly degraded hydrocellulose he has shown that the only glycosidic bonds readily available fo hydrolysis are in the amorphous regions, even those in the surface of the crystallites being very resistant to acid attack. When all th amorphous material has been dissolved degradation proceeds ver slowly by an end-wise attack on the crystallites, single molecules c glucose being severed successively from the ends of the chain molecules.

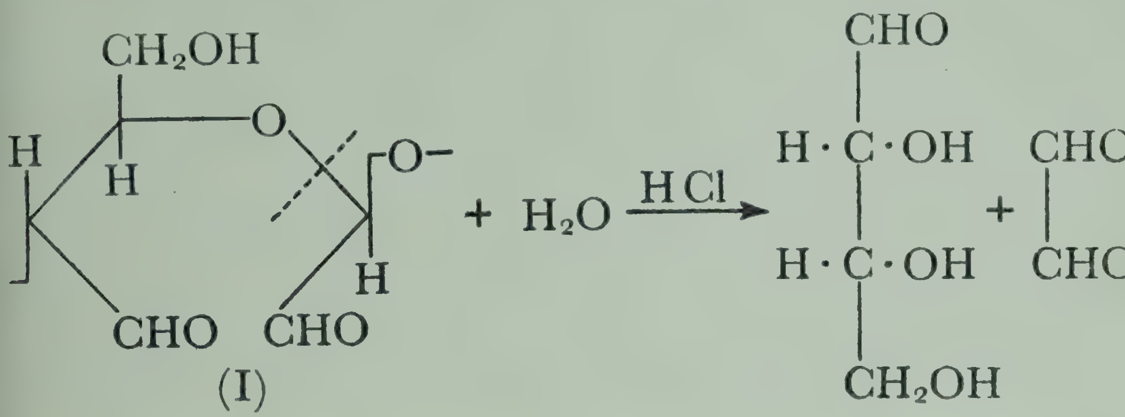
OXYCELLULOSES

The unspecific nature of the reaction of most oxidizing agents wit cellulose, and the virtual confinement of their action to the amorphous parts of the material, make the general study of oxycellulose very difficult. The most important advances in this field hav therefore come from investigations with two oxidizing agents whos mode of attack upon cellulose has been found to be reasonabl specific and which are able to react with both the amorphous an crystalline regions of the material without destroying its fibrou form. These two oxidants are periodic acid (and periodates) an nitrogen dioxide. The results obtained with them are valuabl because they can be applied to the interpretation of the mor complicated results found with industrially important oxidants suc as hypochlorite, chromic acid, and the like.

Periodate Oxycelluloses

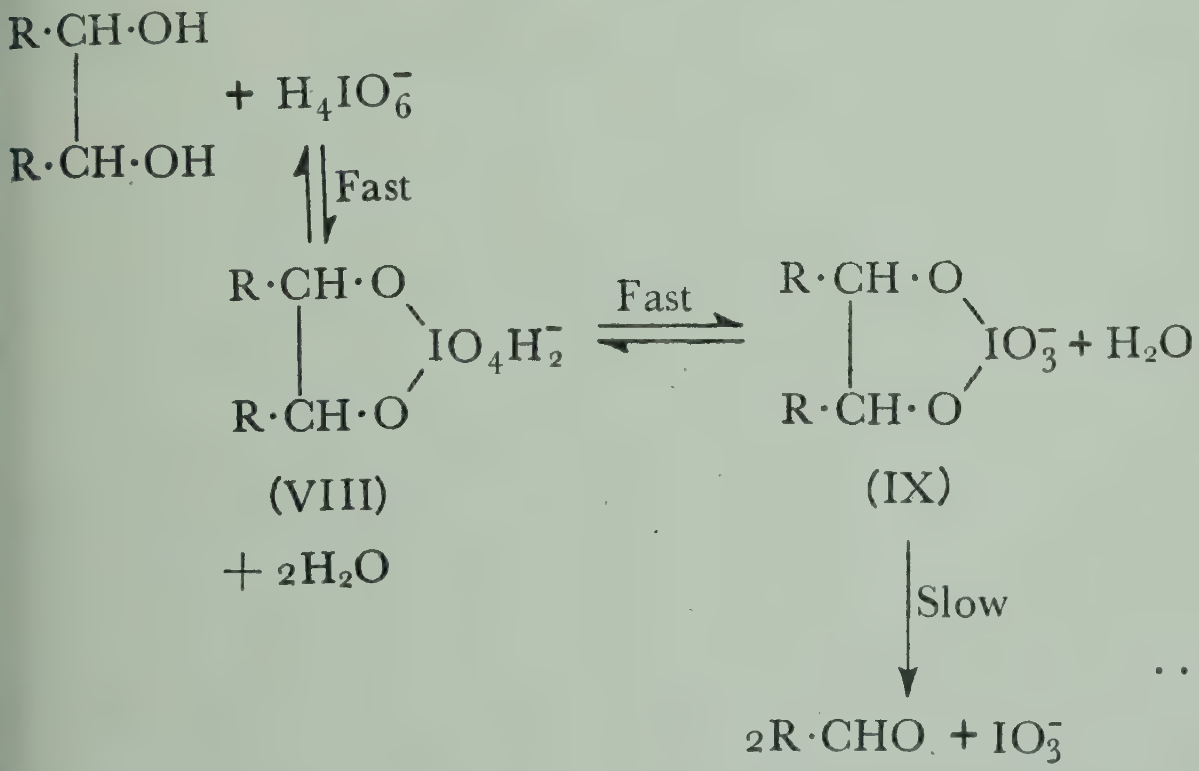
Cellulose was first oxidized with periodic acid by Jackson an Hudson,[35] who showed that the reaction followed the norm

Malaprade course, each chain-unit being converted to a 2 : 3 dialdehyde unit (I). Hydrolysis of the oxycellulose yielded glyoxal, which was identified as its *bis*phenylhydrazone, and D-erythrose, which was identified by oxidizing it to D-erythronic acid with bromine water and isolating the brucine salt:


CH₂OH
O
H
H
O–
H
CHO CHO
(I)
+ H₂O
HCl
CHO
H·C·OH
H·C·OH
CH₂OH
+
CHO
CHO

. . . (10)

The mechanism of the oxidation of cellulose by periodate has recently been shown to be the same as that generally postulated for the oxidation of simple α-glycols in solution.[36] It consists of the rapid formation of a univalent anionic ester (VIII), which is readily dehydrated to (IX). Structure (IX) decomposes slowly and


R·CH·OH
R·CH·OH
+ H₄IO₆⁻
Fast
R·CH·O
R·CH·O
IO₄H₂⁻
(VIII)
+ 2H₂O
Fast
R·CH·O
R·CH·O
IO₃⁻ + H₂O
(IX)
Slow
2R·CHO + IO₃⁻

. . . (11)

unimolecularly to the final reaction products by an electronic process similar to that occurring in intramolecular rearrangements. Striking evidence in support of this scheme in the case of cellulose has been obtained by studying the separate effects of temperature, added electrolyte, and pH on the rate of the reaction in its early stages. Figure 6 shows the effect of temperature on the rate of oxidation of cotton by unbuffered 0·01M sodium metaperiodate. At 20°, the rate curve shows no discontinuity, but at 0° there is an initial fast reaction, identifiable with the formation of the complex (VIII) or (IX), followed by oxidation proceeding at a lower, and constant, rate. The extent of the initial rapid reaction may be estimated by extrapolating the linear portion of the curve to zero time, as indicated by the broken line. Copper number measurements have confirmed that the periodate consumption so obtained is not accompanied by any oxidation of the cellulose. The non-oxidative consumption of periodate from a 0·005M solution is lower than from a 0·01M solution, as would be expected (see Figure 6).

If the oxidation of cellulose by periodate proceeds by way of a charged intermediate the effect of an added electrolyte, e.g. sodium chloride, should be predictable, at least qualitatively, from Donnan's

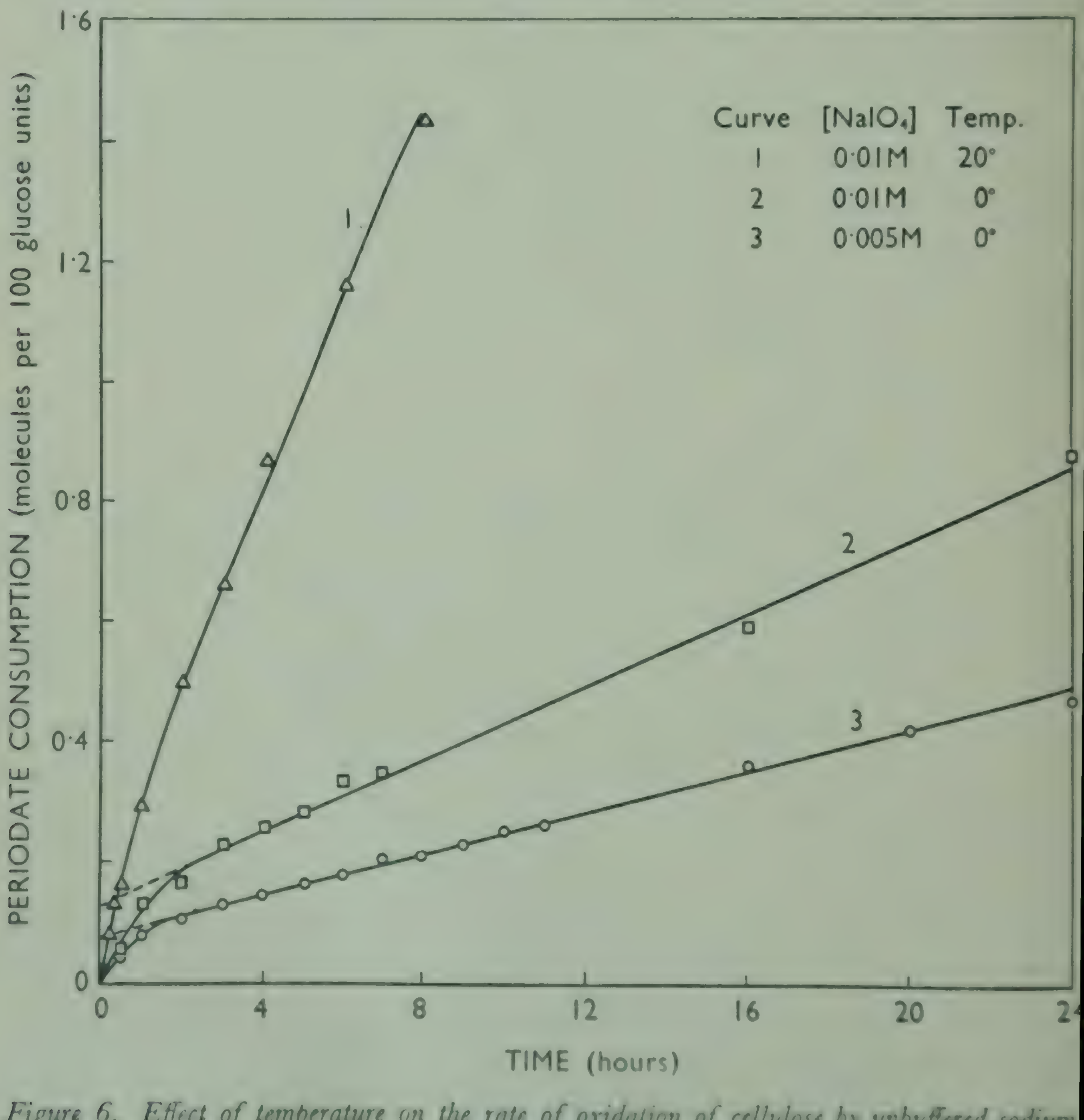


Figure 6. Effect of temperature on the rate of oxidation of cellulose by unbuffered sodium metaperiodate[30]

theory of membrane equilibrium. The system consists of a liquid phase containing ‘free’ periodate ions and a cellulose phase containing both free periodate ions and the non-diffusible anions (VIII) and (IX). The distribution of free periodate ions between the two phases is determined by the total amount of diffusible electrolyte in the system. In particular, the concentration of free periodate in the cellulose phase is less than the concentration in the liquid phase, but can be increased by the addition of an inert, diffusible, salt, such as sodium chloride. Such an increase must inevitably lead to an increase in the amount of periodate bound as an ionic complex, and therefore to an increase in the rate of oxidation. Figure 7 shows that this is precisely the effect of adding sodium chloride to the reaction mixture when cellulose is oxidized by 0·01M sodium metaperiodate at 20°. The addition of salt to

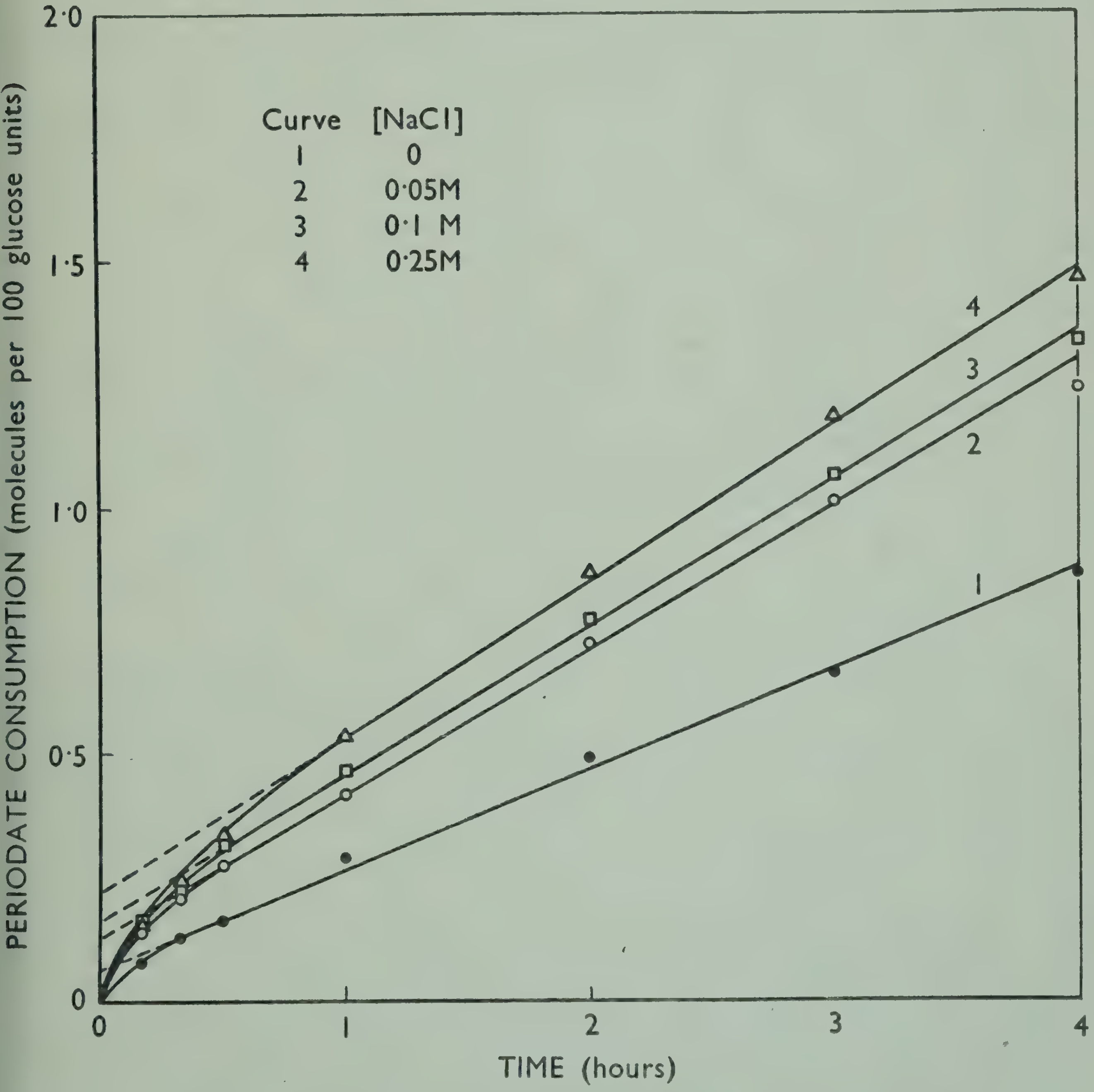
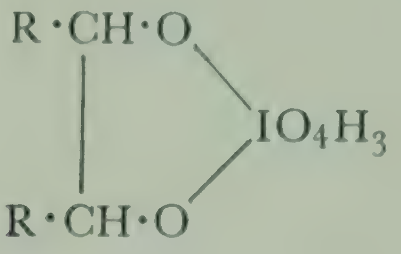
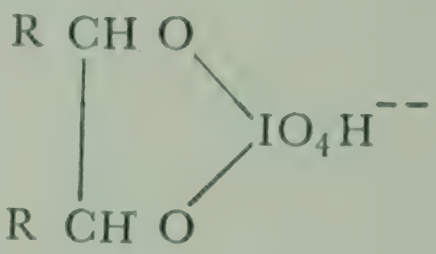


Figure 7. Effect of sodium chloride on the rate of oxidation of cellulose by unbuffered sodium metaperiodate[36]

this system is exactly analogous to its use in the dyeing of cellulos with a direct dye. The only difference between the two systems i that the complex of the cellulose with the dye anion is stable whereas that with the periodate anion is not.

The general effect of pH on the rate of the reaction is the same a that found for the oxidation of simple α-glycols in homogeneou solution. It reflects the fact that only the singly-charged comple (VIII) can be dehydrated to give a structure of type (IX) that i able to decompose directly by electronic rearrangement. Thus th reaction rate passes through a broad maximum over the pH range 2–7, within which the predominating ionic species are singly-charged. In more acid and more alkaline solutions, where the predominating species are the uncharged complex (X) and the doubly-charged complex (XI) respectively, the rate is greatly reduced.


(X)


(XI)

Neither of these structures can decompose in the simple way possible for structure (VIII). At pH 10 the rapid non-oxidative consumption of periodate is much greater than in the unbuffered solutions. The subsequent oxidation is extremely slow, however, and is to be attributed to the small amount of singly-charged complex present even at this pH rather than to a slow decomposition of the doubly-charged species.

The properties of periodate oxycelluloses were exhaustively studied by Davidson.[37] Most notable among these properties are high copper-reducing power and extreme alkali-sensitivity (see Figure 1). Both these characteristics are attributable to the aldehyde groups in the oxidized chain-units. When these aldehyde groups are oxidized to carboxyl groups by means of chlorous acid, the reducing power and alkali-sensitivity are virtually eliminated in the case of oxycelluloses of low aldehyde content, and greatly reduced in the case of more highly oxidized materials.[6] This is illustrated in Table 1, which shows the effect of treating a series of periodate oxycelluloses with chlorous acid under the most favourable conditions. The large reductions of fluidity demonstrate the loss of alkali-sensitivity. The high fluidities of the original oxycelluloses are due to alkaline degradation in the cuprammonium solution and not to chain-scission during the periodate oxidation; the low fluidities of the chlorite-treated materials (for convenience called periodate–chlorite oxycelluloses) show that the double treatment with periodate and chlorous acid causes very little chain-scission, and yields a product that is stable to alkali. As the degree of periodate oxidation increases, the reduction of fluidity effected by treatment with chlorous acid becomes less striking owing to an increase in the proportion of glycosidic linkages severed during the treatment. The reducing power and alkali-sensitivity of periodate oxycelluloses may also be eliminated by reducing the aldehyde groups to primary alcohol groups with sodium borohydride.[38]

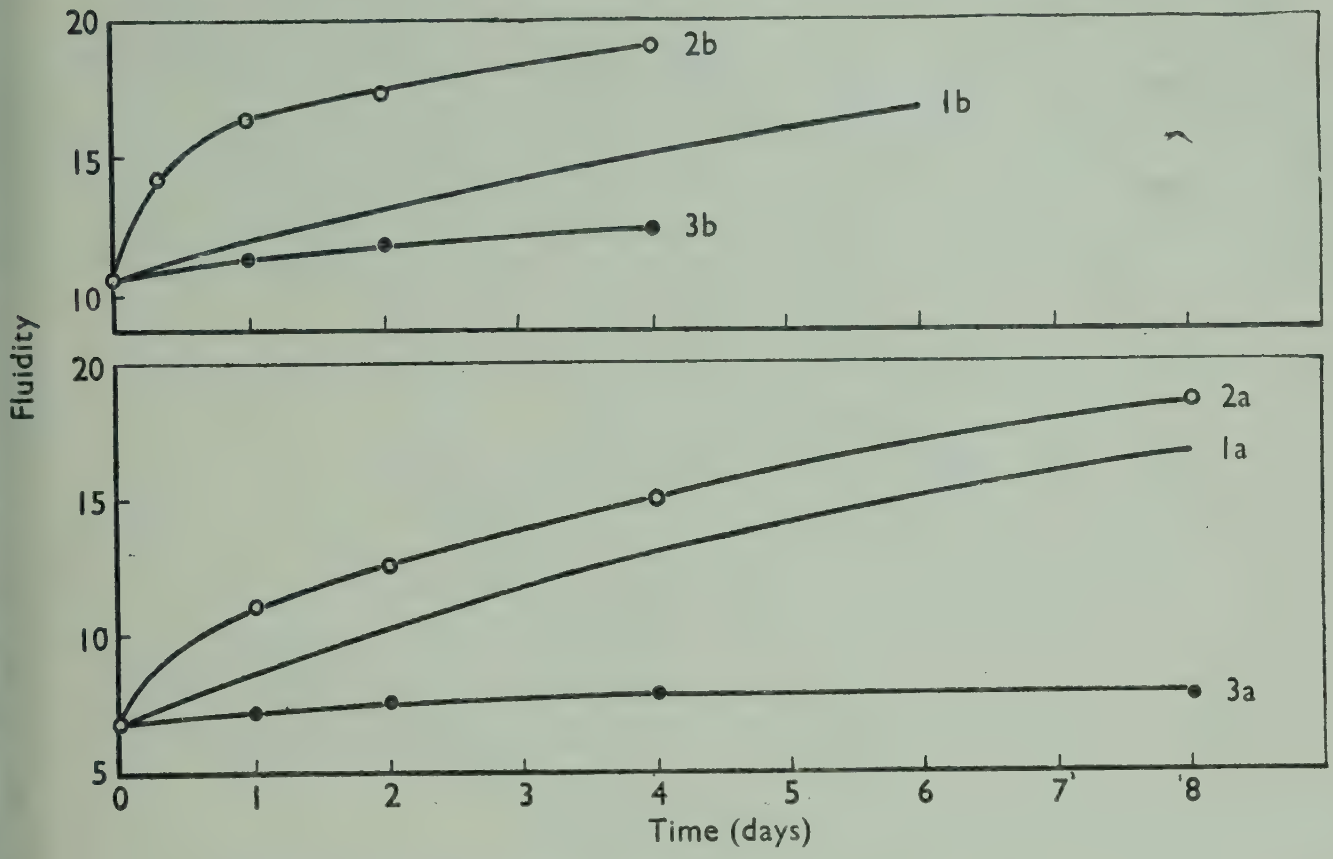
Table 1: Effect of chlorous acid on periodate oxycelluloses[6]

Periodate consumption (molecules per glucose unit)	Copper number		Fluidity		Carboxyl content (millimoles/100 g)		Yield of carboxyl (per cent)
	Initial	Final	Initial	Final	Initial	Final	
0	<0·005	—	3·0	—	0·53	—	—
0·0037	1·14	0·005	25·8	5·5	0·52	3·90	74
0·0074	2·34	0·01	35·9	7·0	0·53	7·50	76
0·0110	3·57	0·02	41·1	9·0	0·56	11·25	79
0·0151	4·77	0·06	44·0	10·7	0·57	15·25	79
0·0187	5·75	0·10	45·5	11·9	0·54	19·3	82
0·0341	9·27	0·25	50·8	20	0·52	38·2	90
0·0452	12·65	0·35	51·5	22	0·60	48·5	87
0·0545	15·9	0·40	52·2	21	0·52	59·5	89
0·072	20·4	0·5	53·5	26	0·48	79	90
0·096	26·9	0·7	54·6	36	0·51	102	87
0·113	29·3	0·8	56·2	35	0·50	124	90
0·146	34·5	0·8	55·6	35	0·49	156	89
0·182	40·1	1·0	58·1	40	0·57	199	91
0·218	44·6	1·2	58·6	42	0·65	232	89
0·245	48·6	1·4	58·5	44	0·73	263	91
0·273	53·8	1·6	62·0	47	0·91	274	85

The last column in the table shows the percentage yield of carboxyl groups calculated on the assumption that every molecule of periodate consumed during the original oxidation gives rise to two carboxyl groups in the final product. The yield rises from 74 per cent for materials of very low degrees of oxidation to a constant value of about 89 per cent for materials in which more than about 3 per cent of the chain-units have been oxidized. Several reasons have been put forward to explain why a 100 per cent yield of carboxyl groups has never been obtained. In the first place, no allowance has been made for the amount of non-oxidative periodate consumption (see page 92) in calculating the yields in the table. Since this is very small, its effect would be negligible except in the case of the very slightly oxidized materials—the materials which, in fact, show the lowest yields of carboxyl groups. Other possibilities are that side reactions accompany glycol-scission even in the early stages of periodate oxidation, and that some dissolution of material richer in carboxyl groups than the insoluble residue occurs during the chlorite treatment. Furthermore, the carboxyl contents of the chlorite-treated materials, being derived from measurements of methylene blue absorption, may be too low owing to autohydrolysis, in spite of efforts made to avoid it. The phenomenon of autohydrolysis, and its effect on carboxyl-content determinations, will now be explained.

When acidic oxycelluloses are stored in the free-acid form and in the air-dry condition (i.e. containing from 5 to 10 per cent of adsorbed water) at ordinary temperatures they exhibit progressive increases of cuprammonium fluidity and of copper number.[39,40] These changes are the result of hydrolytic scission of glycosidic linkages under the influence of hydrogen ions derived from the carboxyl groups. They occur more rapidly the higher the temperature and the greater the amount of adsorbed water in the oxycellulose. Conversely, they can be very nearly completely arrested either by reducing the temperature to 0° or by removing all the adsorbed water by evacuation over phosphorus pentoxide. As would be expected, neutralization of the carboxyl groups with sodium or other cations prevents autohydrolysis altogether. It appears that glycosidic linkages adjacent, or at least near, to chain-units containing carboxyl groups are hydrolysed preferentially, because short-chain carboxylic acids may be washed out with water from materials that have undergone autohydrolysis. These short-chain acids have soluble methylene blue salts, so that, when a periodate–chlorite oxycellulose that has suffered autohydrolysis is treated with a buffered methylene blue solution, the fall in concentration of the dye corresponds only to the carboxyl content of the insoluble fraction of the material, and not to its total carboxyl content.

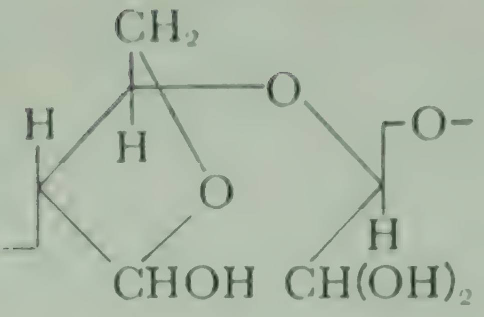
The glycosidic linkages adjacent to chain-units containing carboxyl groups are not only hydrolysed preferentially (an effect that may be due to a local concentration of hydrogen ions higher than in the rest of the material), but they are also more susceptible to hydrolysis. This is evident from Figure 8 which compares the rate of increase of cuprammonium fluidity of two periodate–chlorite oxycelluloses (having carboxyl contents of 6·3 and 14·8 millimoles per 100 g respectively) with that of two hydrocelluloses with the same respective initial fluidities, on hydrolysis with N-hydrochloric acid at 20°. The fluidities of the oxycelluloses rise much more rapidly than those of the hydrocelluloses in the early stages of the acid treatment, but subsequently the two rates become equal. The effect of water on the fluidities of the two oxycelluloses is also shown in Figure 8. It demonstrates that autohydrolysis makes only a very small contribution to the total hydrolytic effect observed during the treatments with N-hydrochloric acid.



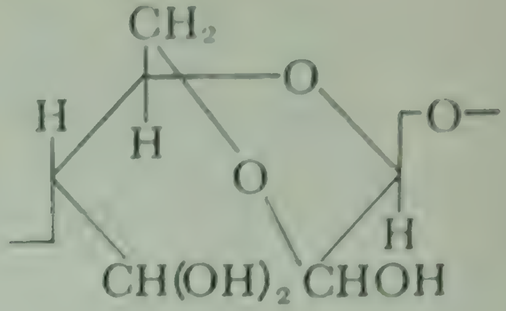
Curves 1a and 1b	Hydrocelluloses; N hydrochloric acid
Curves 2a and 2b	Periodate-chlorite oxycelluloses; N hydrochloric acid.
Curves 3a and 3b	Periodate-chlorite oxycelluloses; water

Figure 8. Rates of hydrolysis of periodate-chlorite oxycelluloses and of hydrocelluloses at 20° [40]

The chemical reactions of periodate oxycelluloses that have been described above are all explicable in terms of the dialdehyde structure (I). However, the infra-red spectrum of these materials contains no absorption band in the region usually associated with carbonyl groups.[41] Various explanations of this have been offered, that most generally accepted being the formation of a cyclic hemi-acetal between one of the aldehyde groups and the primary alcohol group, together with hydration of the other aldehyde group, as in formulae (XII) and (XIII). A structure containing one free aldehyde group is supported by Mester's observation[42] that only one diphenylformazan group per dialdehyde chain-unit can be

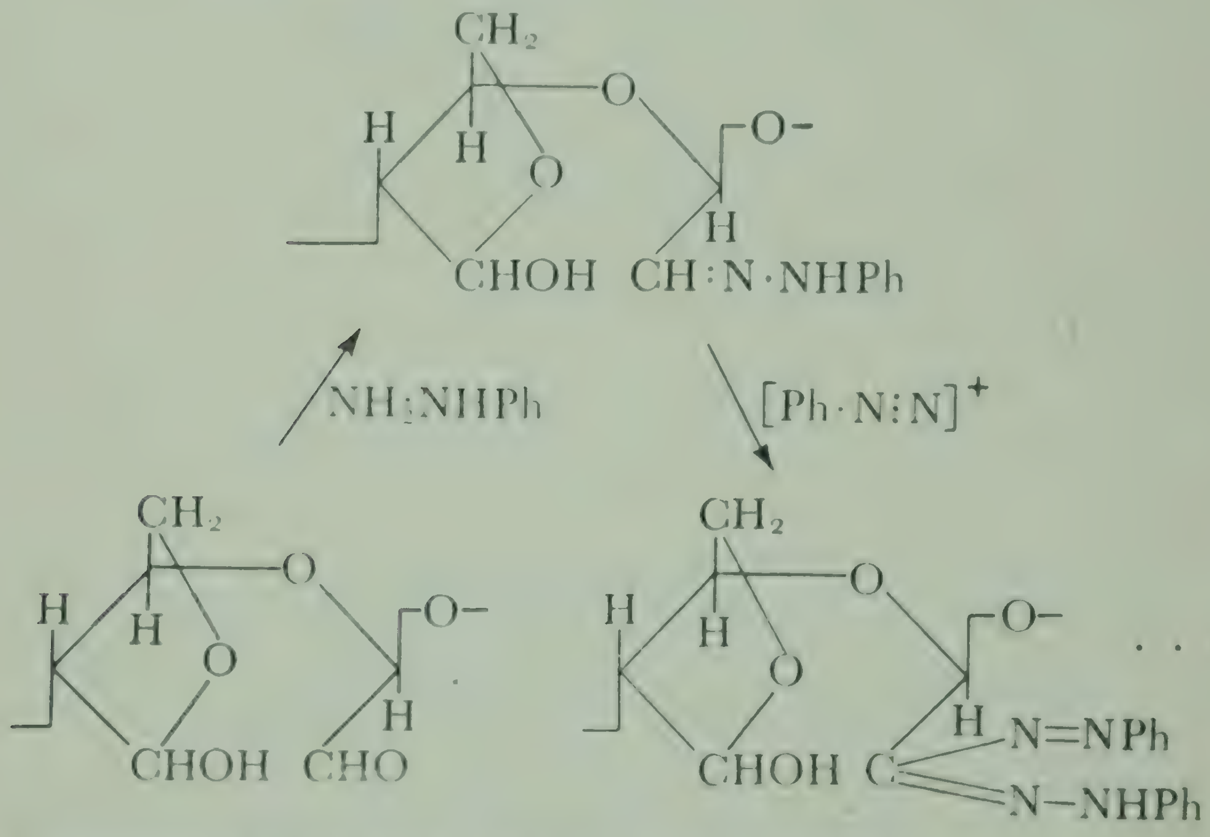


(XII)



(XIII)

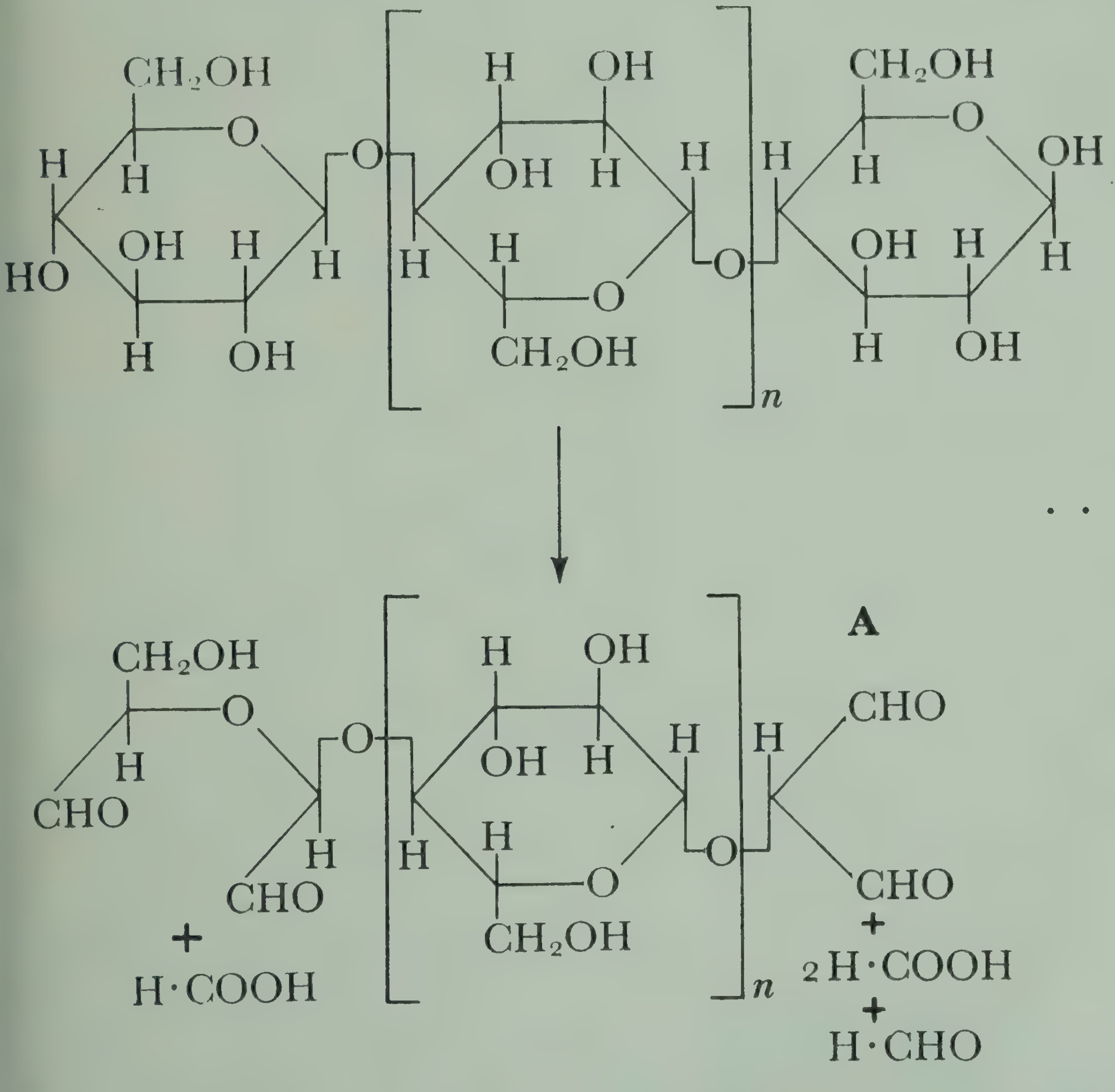
introduced into periodate oxycelluloses by condensing them with phenylhydrazine and allowing the products to react with benzene diazonium chloride in the presence of pyridine. Mester assumes that the unhydrated form of (XII) takes part in this series of reactions, which may therefore be formulated



. . . (12)

Structures (XII) and (XIII) are insufficient by themselves to explain the effect of diazomethane on periodate oxycelluloses.[43] This reagent methylates periodate oxycelluloses to the extent of introducing 1 methoxyl group per chain-unit. Simultaneously it eliminates both their reducing power and their alkali-sensitivity. Structures (XII) and (XIII) are in accord with the introduction of 1 methoxyl group per chain-unit, but do not explain the loss of reducing power and alkali-sensitivity unless it is further assumed that the free aldehyde group reacts with diazomethane with the formation of an ethylene oxide derivative. An alternative explanation of the diazomethane results is that hemi-acetal formation takes place between adjacent chain-molecules, thus leading to a cross-linked product. The insolubility in acetone of the nitrates of periodate oxycelluloses containing more than 2 per cent of oxidized chain-units may be explained on this basis. The significance of the various possible structures of periodate oxycelluloses in relation to the effects of diazomethane treatment have been fully discussed by Head.[43]

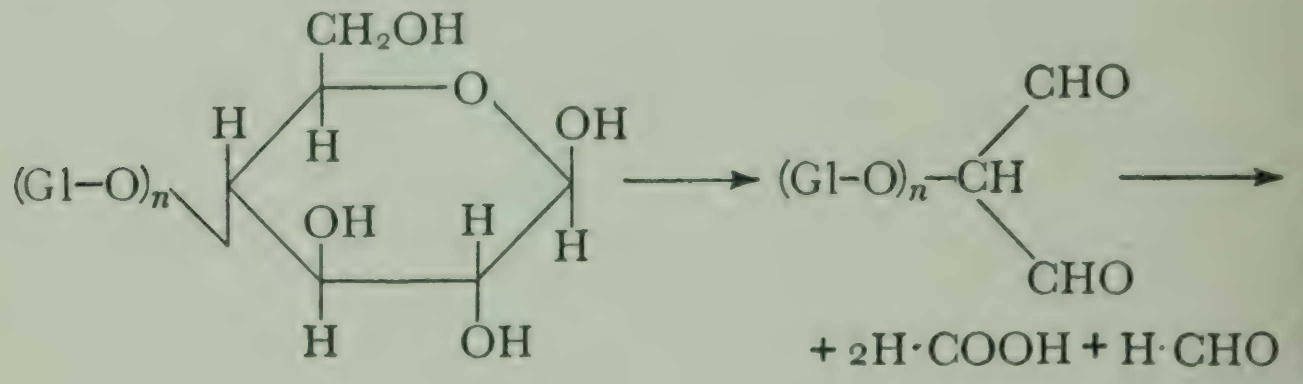
In the above account of the oxidation of cellulose by periodate the reactions at the ends of the chains have not been considered. If these reactions are solely of the Malaprade type, and if the formate ester initially produced at the reducing end of the chain is sufficiently readily hydrolysed, each chain-molecule of cellulose should give rise to three molecules of formic acid and one of formaldehyde as shown in equation (13).



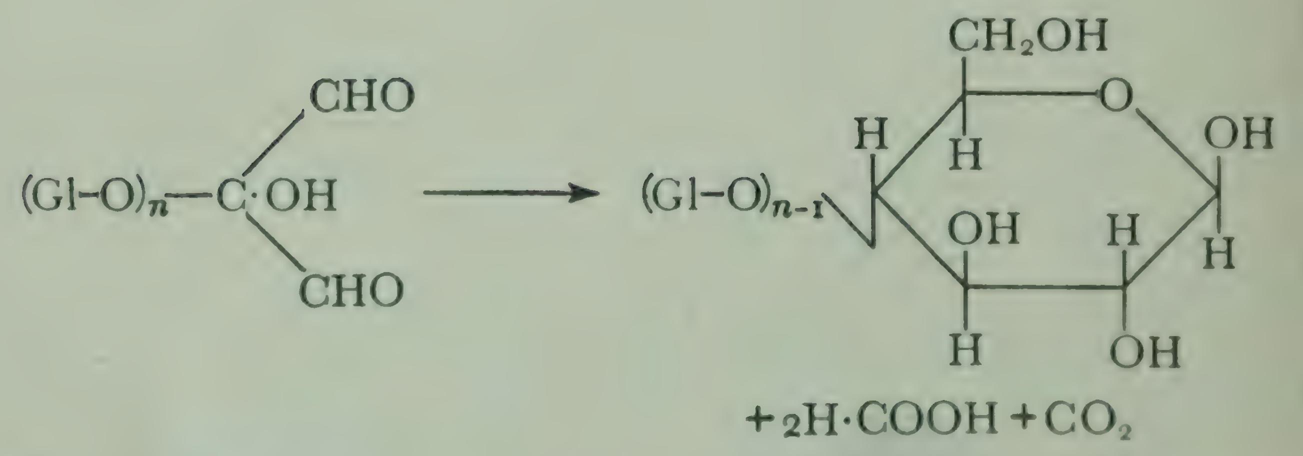
. . . (13)

This is the basis of the determination of the chain-length of cellulose from the yield of formic acid on oxidation with periodate. Most workers who have used the method have obtained absurdly low results owing to the formation of extra formic acid by over-oxidation. Hirst and his collaborators,[44] however, have obtained a chain-length of at least 1,000 using particularly dilute solutions of periodate under

conditions that give the theoretical yield of formic acid from simpl
glycosides.[45] They do not, however, report any evidence either th
the time allowed for the reaction is sufficient for the complet
oxidation of the end-groups according to equation (13), or that th
production of extra formic acid by over-oxidation can be prevente
Head[46] has pointed out that the group **A** in equation (13) should b
susceptible to further periodate oxidation. He has also shown[47] tha
cellobiose, which is a model substance for cellulose, is oxidized t
nine molecules of formic acid, two molecules of formaldehyde, an
one molecule of carbon dioxide. It can therefore be concluded tha
the group **A** is, in fact, oxidized to two molecules of formic acid an
one of carbon dioxide. Thus a reducing end-group in cellulos
probably gives four molecules of formic acid on oxidation wit
periodate, together with a new reducing end-group, as shown i
equation (14).



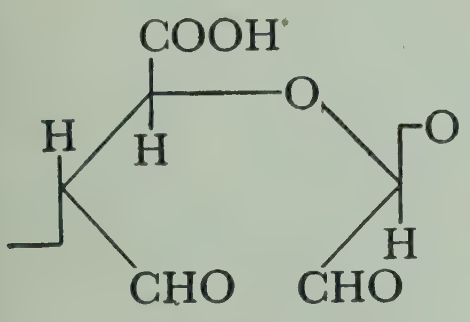
. . . (14)



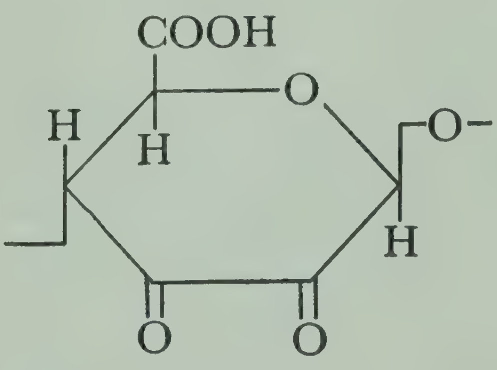
The new reducing end-group can then be oxidized by the sam
mechanism, which therefore leads to the progressive scission of th
chain-molecules from their reducing ends, and the continuou
production of formic acid. The oxidation of the chain-unit adjacen
to group **A** to a 2 : 3 dialdehyde unit before the oxidation of group **A**
does not affect these conclusions. Further work is therefore necessar
to determine whether the periodate method can be validly applie
to the measurement of the chain-length of cellulose.

Nitrogen Dioxide Oxycelluloses

The main reaction that occurs when cellulose is allowed to react with gaseous nitrogen dioxide is oxidation of the primary alcohol groups to uronic acid groups. This has been demonstrated by uronic acid determinations by the method of Lefèvre and Tollens.[48,49,50] The properties of the products are very different from what would be expected if this were the only reaction, however. In particular, nitrogen dioxide oxycelluloses possess high copper numbers and are extremely alkali-sensitive. This implies that other types of oxidation play an important part in their formation. Furthermore it has been shown[50] that the amount of oxygen consumed in the production of a nitrogen dioxide oxycellulose is greater than that required to produce the uronic acid groups it contains. For example, the conversion of each primary alcohol group in cellulose to a carboxyl group requires two atoms of oxygen; nevertheless, in the formation of an oxycellulose containing 1 carboxyl group per chain-unit, 2·35 atoms of oxygen per chain-unit are consumed. The extra 0·35 atom of oxygen per chain-unit represents the extent to which side reactions occur. The side reactions that most readily explain the reducing power and alkali-sensitivity of the oxycelluloses, and for which definite evidence exists, are oxidation of the 2 : 3 glycol group to a dialdehyde (as in periodate oxidation) and to a diketone. In highly oxidized materials these groups must be formed in chain-units whose primary alcohol group has also been oxidized, thus giving structures (XIV) and (XV) respectively.

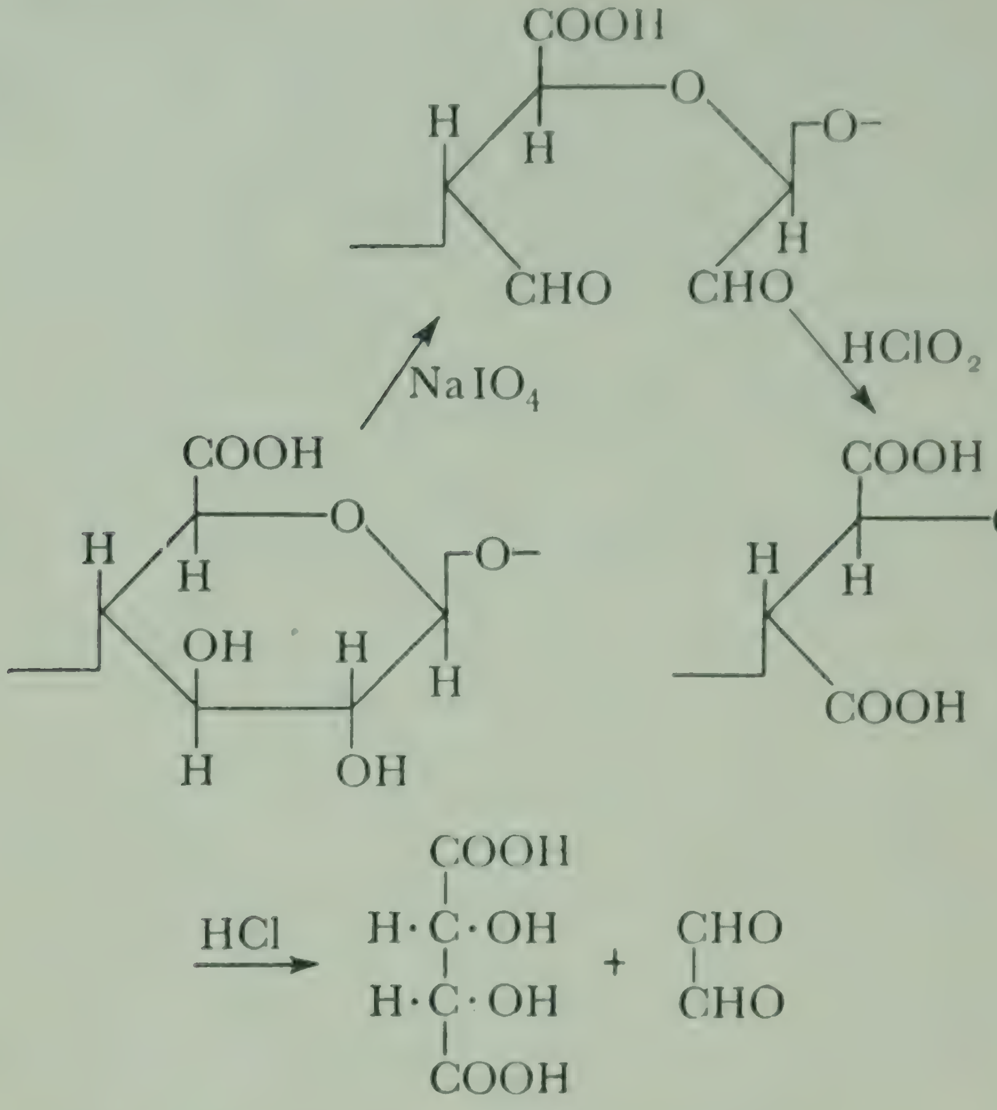


(XIV)



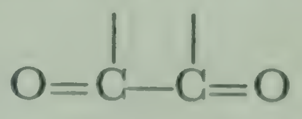
(XV)

Direct evidence that the oxidation of cellulose by nitrogen dioxide takes place mainly at the carbon atom in position 6 is afforded by Head's isolation of *meso*tartaric acid (identified as the brucine salt) from the mixture obtained by treating a highly oxidized material with periodate and chlorous acid, and hydrolysing the product with acid.[51] This series of reactions may be represente by equation (15).



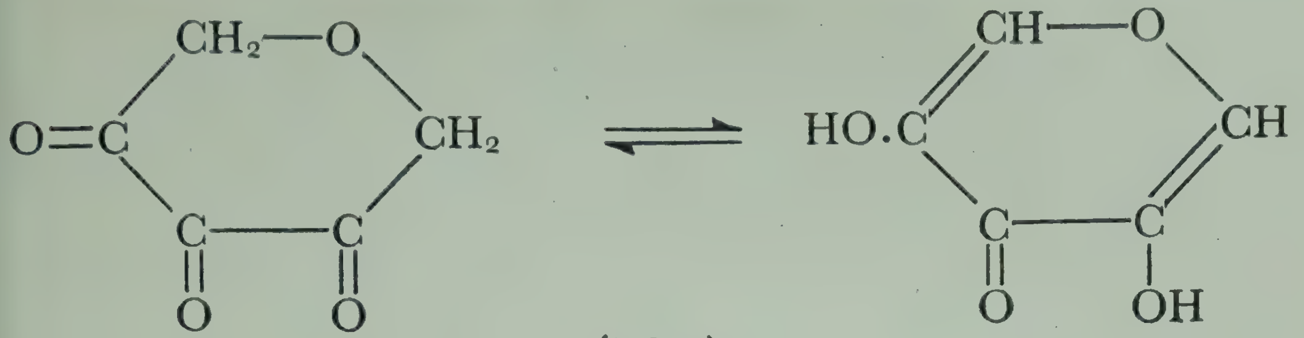
. . . (15)

Evidence for the existence of dialdehyde chain-units in nitrogen dioxide oxycelluloses includes the increase of carboxyl content effected by treating them with chlorous acid[50] and their strong affinity for methanol.[52] The presence of diketone groups has been inferred from the lemon-yellow colour acquired by highly oxidized materials when thoroughly dried, most diketones being yellowish in colour. The fact that the air-dry materials, which contain from 20 to 25 per cent of adsorbed water, are colourless may be ascribed to the destruction of the chromophoric character of the



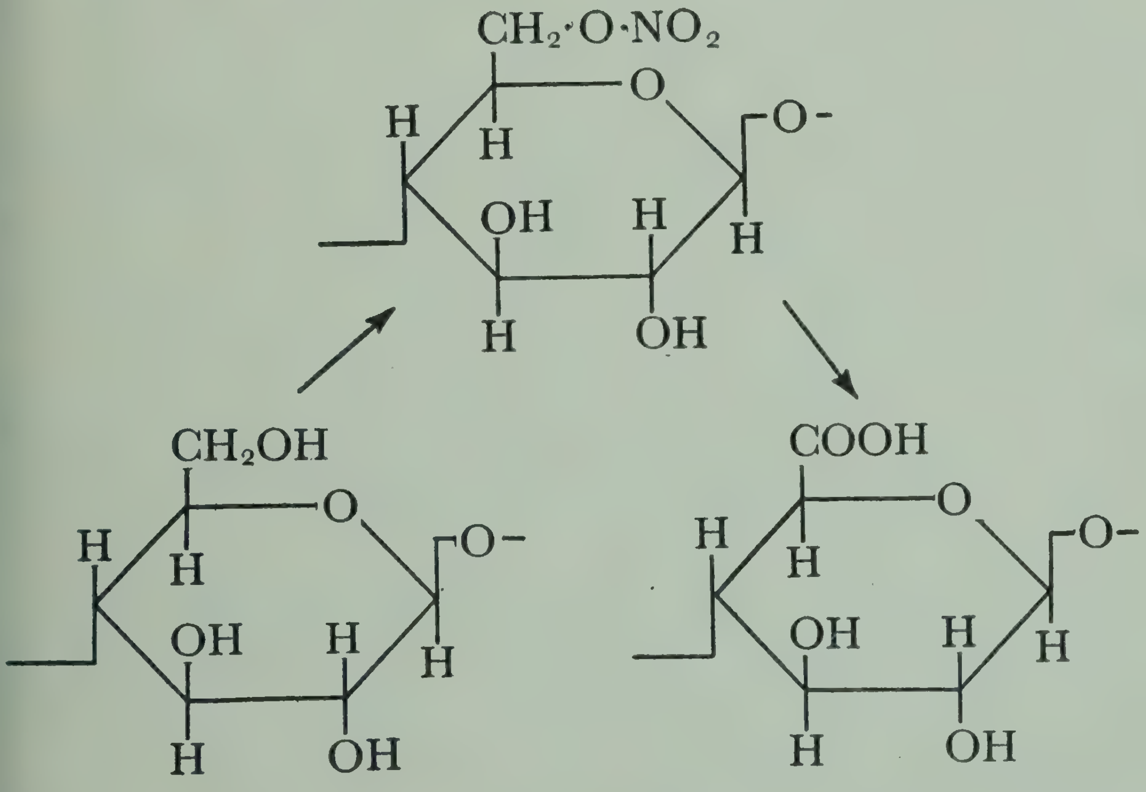
$$\mathrm{O{=}\overset{|}{C}{-}\overset{|}{C}{=}O}$$

group by hydration. Battenberg and Berg[53] claim to have obtained small quantities of 3 : 5-dihydroxy-γ-pyrone (XVI) by the careful hydrolysis of nitrogen dioxide oxycelluloses, thus proving the presence in them of chain-units of type (XV). This substance would arise from the decarboxylation and intramolecular dehydration o the monomer originating from these chain-units.



(XVI)

The mechanism of the oxidation of the primary alcohol groups in cellulose by nitrogen dioxide is more complicated than would appear at first sight. Kenyon and his collaborators[54] have found that, when the reaction is carried out with a solution of nitrogen dioxide in carbon tetrachloride, the nitrogen content of the product rises rapidly to a maximum and then falls gradually to quite a low value. The carboxyl content rises continuously throughout the reaction, however. This suggests that the first stage of the reaction is the formation of cellulose 6-mononitrate, which is then converted to the corresponding uronic acid by a combined process of denitration and oxidation:



. . . (16)

The precise nature of the species that operate at each stage of the reaction is not clear, but nitric acid must play an essential part, because cellulose nitrates containing up to one nitrate group per chain-unit, but no more, can be prepared by means of suitable mixtures of nitrogen dioxide and anhydrous nitric acid in carbon tetrachloride. Iodination of these materials with sodium iodide in acetonylacetone shows that virtually all the nitrate groups are in position 6, and prolonged treatment of them with solutions of nitrogen dioxide and small quantities of nitric acid in carbon tetrachloride converts them into ordinary nitrogen dioxide oxycelluloses. The evidence for the scheme (16) is strong, but further elucidation of its precise mechanism is desirable.

Among the more interesting properties of highly oxidized nitrogen dioxide oxycelluloses is their behaviour with dilute ammonia.[50] When treated with this reagent they swell to clear, homogeneous, gels that are so stiff that the containing vessel can be inverted without its contents being lost. Within a few minutes the gel liquefies, giving a fluid solution similar to that obtained immediately when a solution of sodium carbonate of about the same pH as that of the ammonia is used. This behaviour provides a striking demonstration of the fact that these fibrous oxycelluloses are composed of long chain-molecules that suffer rapid scission under the action of a weak alkali. The retardation of the scission in the presence of ammonia must be due to some reversible condensation of the ammonia with the carbonyl groups responsible for the alkali-sensitivity.

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4

THE ALKALINE DEGRADATION OF CELLULOSE AND CHEMICALLY MODIFIED CELLULOSE

INTRODUCTION

The alkaline degradation of both cellulose and chemically modified cellulose is of vital importance to all processers and users of cellulose and cellulosic material. It may be a desired reaction of some basic process, e.g. viscose ageing, but more frequently it is an unwanted reaction as in the alkali ‘ tendering ’ of oxidized (e.g. photo-oxidized and bleached) fabrics, or the loss of α-cellulose during the hot alkali refining of wood pulp. Oxidized fabrics essentially retain their appearance and strength until treated with alkaline washing agents when holes develop at the places which have been exposed to oxidation. In the latter case, wood pulp is treated with hot alkali to remove β- and γ-celluloses leaving a pulp of high α-cellulose content suitable for the preparation of cellulose derivatives and of high tenacity fibres. Low yields of purified pulp result from this process because about 20 per cent of the original α-cellulose is degraded and converted into β- and γ-celluloses. Because of the importance to industry of the alkaline degradation of cellulosic material, a considerable amount of effort has been devoted to this subject since the end of the last century. Particular mention must be made of the work of G. F. Davidson who has made considerable progress to the understanding of the reactions of cellulose towards alkali. With the advent of new techniques, particularly chromatography, rapid progress has been made in this particular field of cellulose chemistry, and it is these latest results which will be discussed.

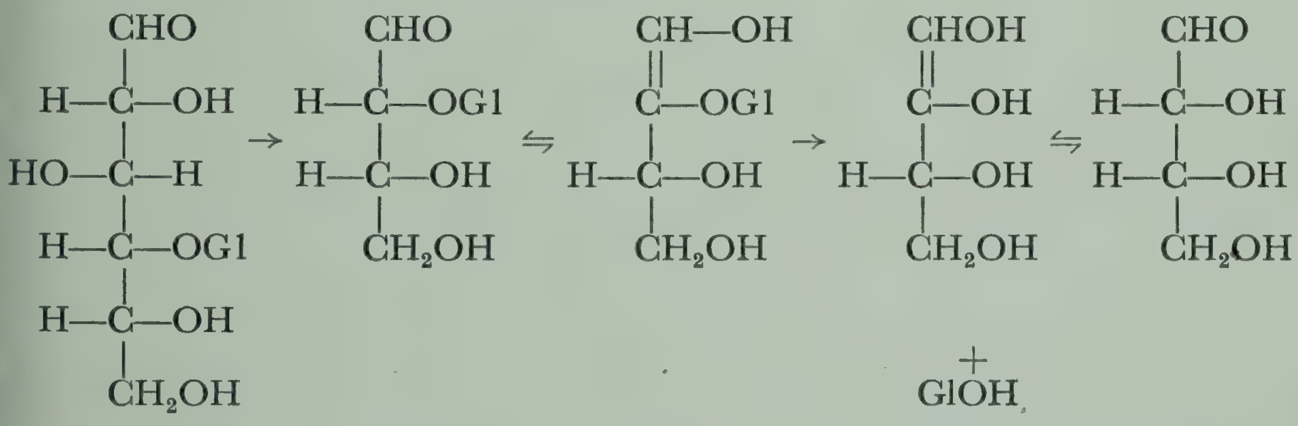
Cellulose itself is slowly attacked by hot alkali and since the weight loss was observed to be a function of the number of aldehydic groups present (i.e. inversely proportional to the degree of polymerization), Davidson[1] suggested that the degradation occurred by a stepwise scission from the reducing end. The importance of the aldehydic groups is illustrated by the fact that if they are removed either by oxidation to a carboxylic acid,[2] reduction to an alcohol,[3] or by glycosidation,[4] the cellulose virtually becomes stable towards alkali. If, however, the number of carbonyl groups is increased by chemical modification such as hydrolysis of the glucosidic linkages with acid or with alkali at high temperatures[5] (170° and above), or by oxidation of either the primary or secondary alcohol groups of the glucose repeating unit to aldehydes or ketones, respectively, then the modified cellulose becomes more labile towards alkali.

Several theories have been proposed to explain the alkaline degradation of oxidized celluloses, but only the following three warrant detailed discussion.

THEORIES OF ALKALINE DEGRADATION OF CARBOHYDRATES

Mechanism of W. L. Evans

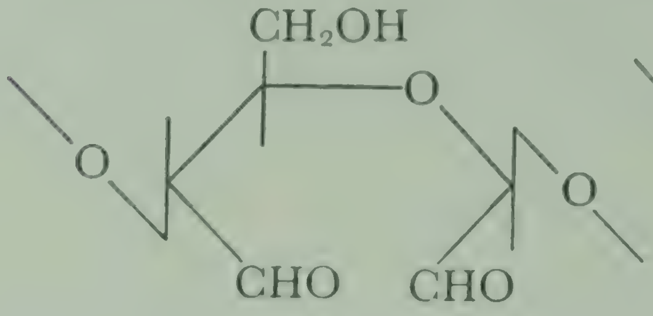
This mechanism is an extension of the work of W. L. Evans and co-workers[6] on the alkaline degradation of oligosaccharides. From a study of the nature of the acidic degradation products of maltose (I, Gl=α-D-glucopyranosyl) it was concluded that a 1 → 4-linked disaccharide undergoes a reversed aldol condensation to give a 2-*O*-D-glycosyl-D-erythrose (II) and formaldehyde and/or glycollic aldehyde (II) is then hydrolysed



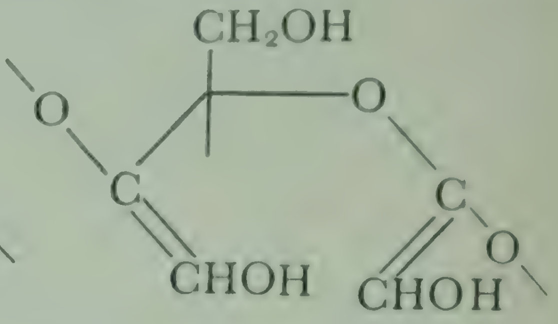
$$\begin{array}{c}\mathrm{CHO}\\ |\\ \mathrm{H{-}C{-}OH}\\ |\\ \mathrm{HO{-}C{-}H}\\ |\\ \mathrm{H{-}C{-}OGl}\\ |\\ \mathrm{H{-}C{-}OH}\\ |\\ \mathrm{CH_2OH}\\ \mathrm{(I)}\end{array} \rightarrow \begin{array}{c}\mathrm{CHO}\\ |\\ \mathrm{H{-}C{-}OGl}\\ |\\ \mathrm{H{-}C{-}OH}\\ |\\ \mathrm{CH_2OH}\\ \mathrm{(II)}\end{array} \leftrightharpoons \begin{array}{c}\mathrm{CH{-}OH}\\ \|\\ \mathrm{C{-}OGl}\\ |\\ \mathrm{H{-}C{-}OH}\\ |\\ \mathrm{CH_2OH}\\ \mathrm{(III)}\end{array} \rightarrow \begin{array}{c}\mathrm{CHOH}\\ \|\\ \mathrm{C{-}OH}\\ |\\ \mathrm{H{-}C{-}OH}\\ |\\ \mathrm{CH_2OH}\\ +\\ \mathrm{GlOH}\\ \mathrm{(IV)}\end{array} \leftrightharpoons \begin{array}{c}\mathrm{CHO}\\ |\\ \mathrm{H{-}C{-}OH}\\ |\\ \mathrm{H{-}C{-}OH}\\ |\\ \mathrm{CH_2OH}\\ \mathrm{(V)}\end{array}$$

(I) (II) (III) (IV) (V)

to glucose and the enediol (IV) of erythrose (V). These two products are capable of further degradation by alkali to give saccharinic, lactic, and other acids. It was postulated that the alkaline hydrolysis occurred through the intermediate enediol (III), the double bond weakening the glycosidic linkage. The results of a study of the alkaline degradation of many carbohydrates were explained by the occurrence of this type of hydrolysis. This mechanism has been used to explain the alkaline degradation of a cellulose molecule in which one of the hydroxyl groups has been oxidized to a carbonyl group.[7] It can also explain the alkali lability of periodate oxidized cellulose (VI) since the enol (VII) has the necessary structure postulated by Evans for alkaline hydrolysis. The products of such a hydrolysis would be the enol of D-erythrose (VIII) together with glyoxal (IX), both of which would undergo further alkaline degradation. Erythrose would be expected to give acids such as the $C_{(4)}$ saccharinic acids and lactic acids, whereas glyoxal would undergo an internal Cannizzaro reaction to give glycollic acid (X).



(VI)



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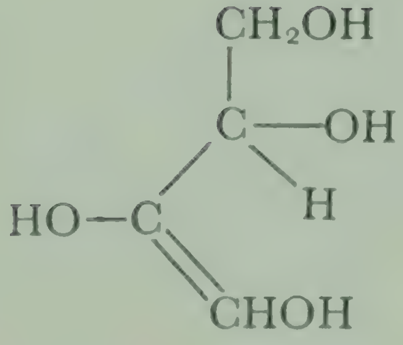
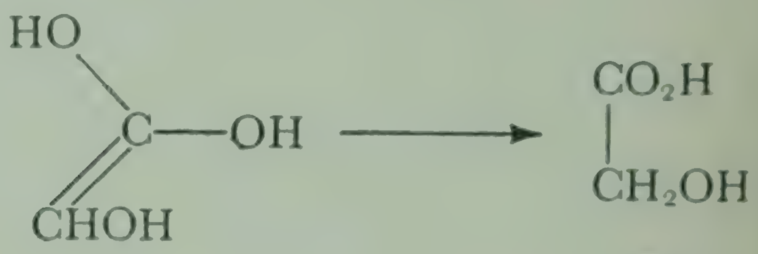


(VIII)

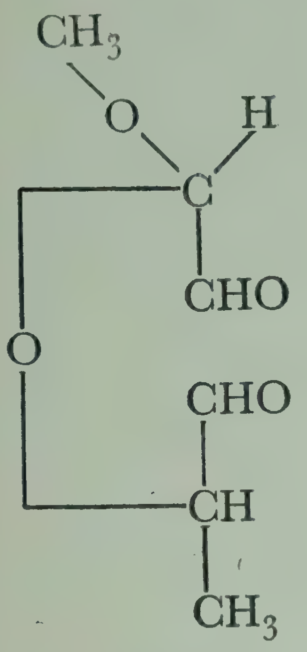


(IX) (X)

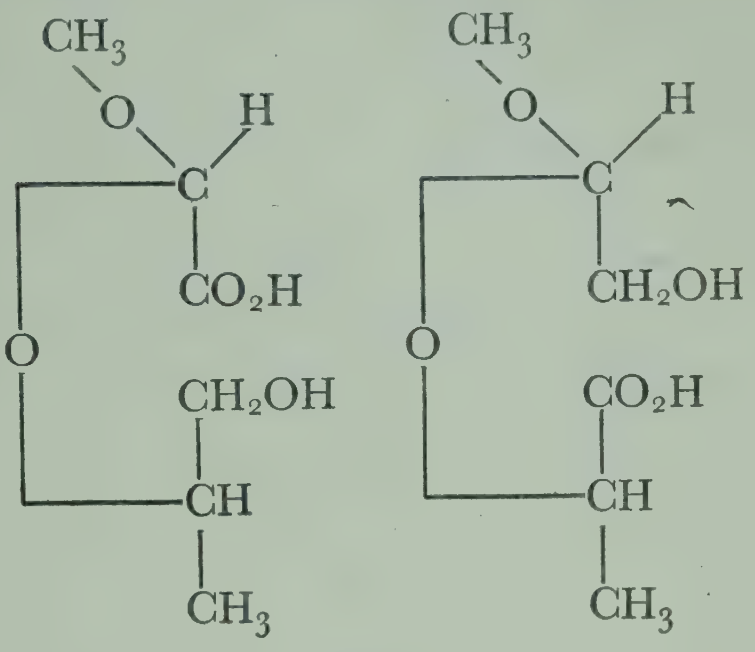
Although this mechanism explains quite simply the alkaline hydrolysis of oligosaccharides and oxidized celluloses, there is no satisfactory evidence that the presence of a double bond in an enediol structure weakens a glycosidic linkage in the α-position.

Mechanism of E. Pacsu

Pacsu in 1945 made a study of the degradation of periodate oxidized cellulose by aqueous sodium hydroxide (1N) at room temperature.[8] Attempts to isolate the acidic products as their brucine salts revealed that the oxidized cellulose had been degraded to acidic products of low molecular weight. Fry, Wilson, and Hudson had previously observed[9] that the periodate oxidation product (XI) of methyl-α-L-rhamnopyranoside consumed exactly one equivalent of alkali on treatment with excess alkali. It was shown that the dialdehyde underwent an internal oxidation–reduction (Cannizzaro reaction) to give a mixture of monobasic acids (XII). Undoubtedly this type of reaction occurs to a certain extent during the treatment of periodate-oxidized cellulose (periodate oxycellulose) with alkali, but the possibility of hydrolysis of the polymer by the resulting acidic products was discounted since the original oxidized polymer was quite stable to hydrochloric

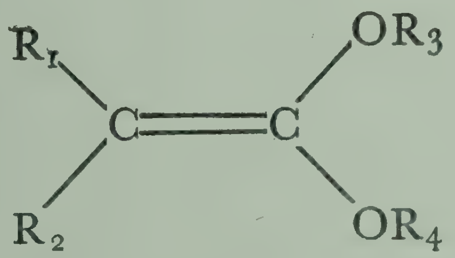


(XI)

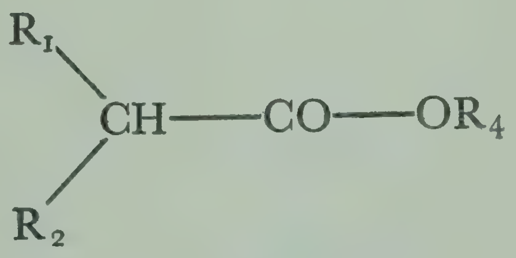


(XII)

acid (2N). Pacsu observed that the enol form of periodate oxycellulose (VII) is very similar in structure to the hydroxyketene acetals (XIII). Negatively substituted hydroxyketene acetals are known to split out 1 mole of alcohol in the presence of alkali, and are ultimately converted into an acetic acid derivative (XIV).

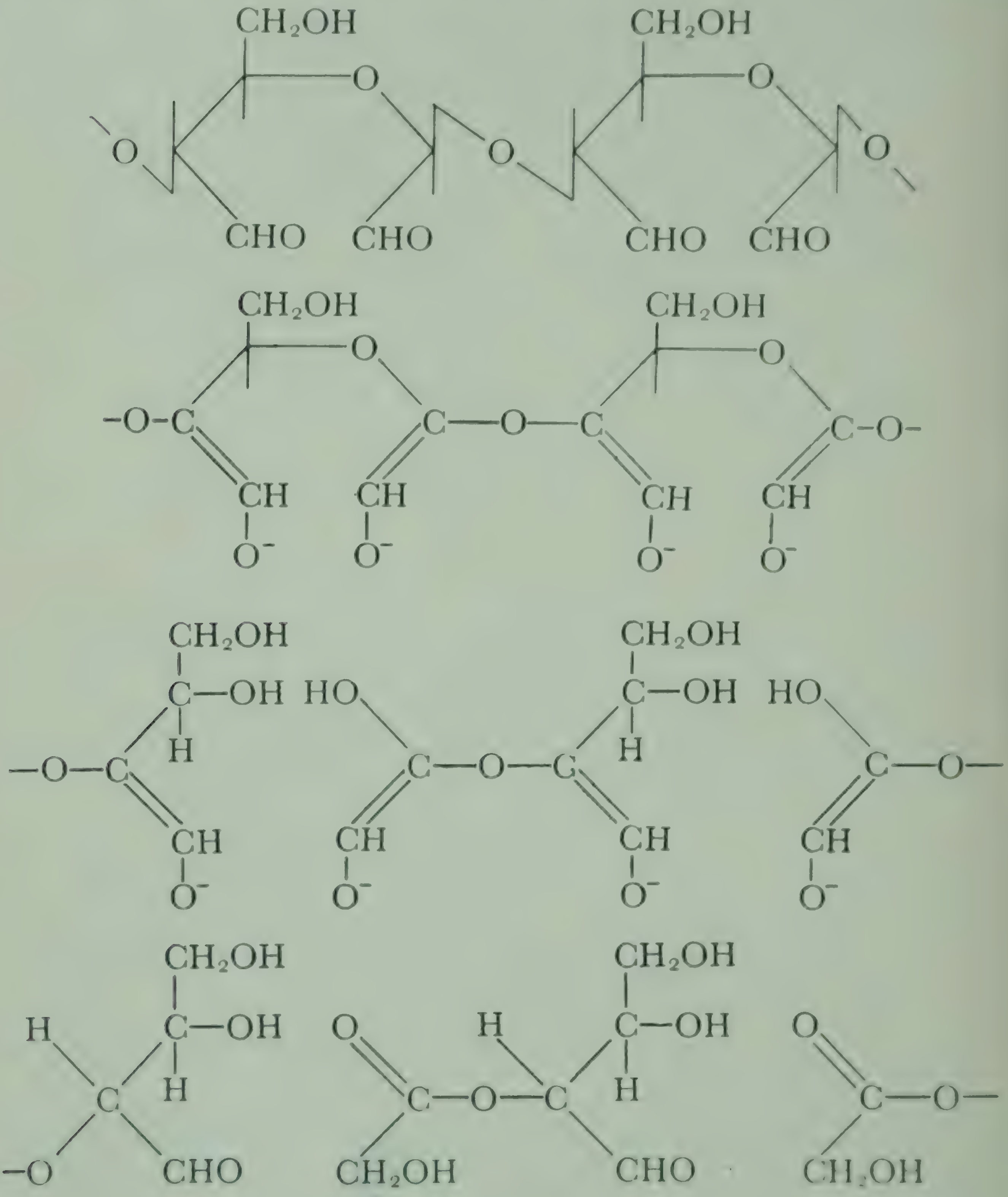


(XIII)



(XIV)

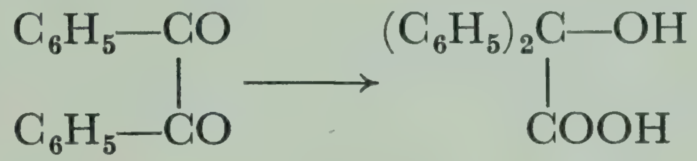
The enol of the oxycellulose was assumed to behave similarly, and the degradation was explained by a three-stage reaction. A proton of the oxidized cellulose is extracted by a hydroxyl ion to give the ion of (VII) which undergoes hydrolysis by an electrophilic attack of water to yield the enol form of glycollic acid ester. Finally, a prototropic change of the enol form of the ester occurs to give the normal form of D-erythrose 2-*O*-glycollate which undergoes alkaline hydrolysis to give the free tetrose and sodium glycollate. The tetrose is capable of further reaction with alkali to give a complex mixture of acids[10] which would make the isolation and identification of the primary and secondary products extremely difficult. The oxidation product (XI) of methyl α-L-rhamnopyranoside apparently undergoes this type of reaction only to a limited extent.



The above mechanism has been advanced by Pacsu to explain the alkaline degradation of all types of oxidized celluloses, but this assumes that all oxidizing agents attack cellulose in a manner similar to that of periodic acid. This is an unwarranted assumption since there is no evidence whatsoever that all oxidations occur by cleavage of glycol groups. Although the postulated mechanism for the alkaline degradation of periodate oxidized cellulose is very attractive, no evidence has been obtained to support it.

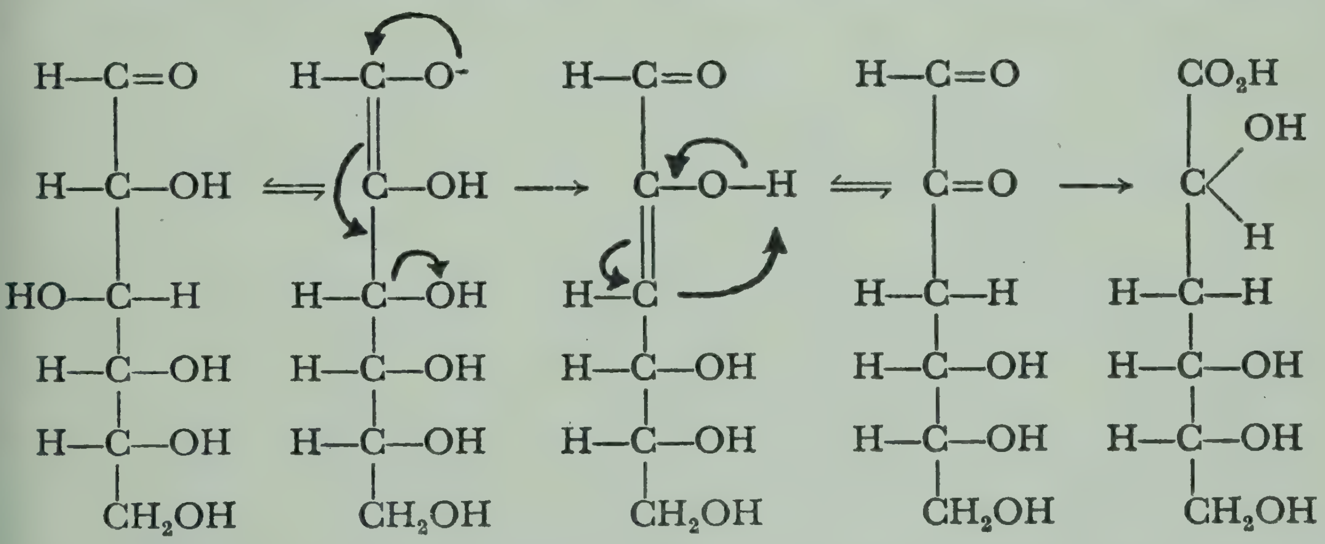
The β-Alkoxycarbonyl Mechanism of H. S. Isbell

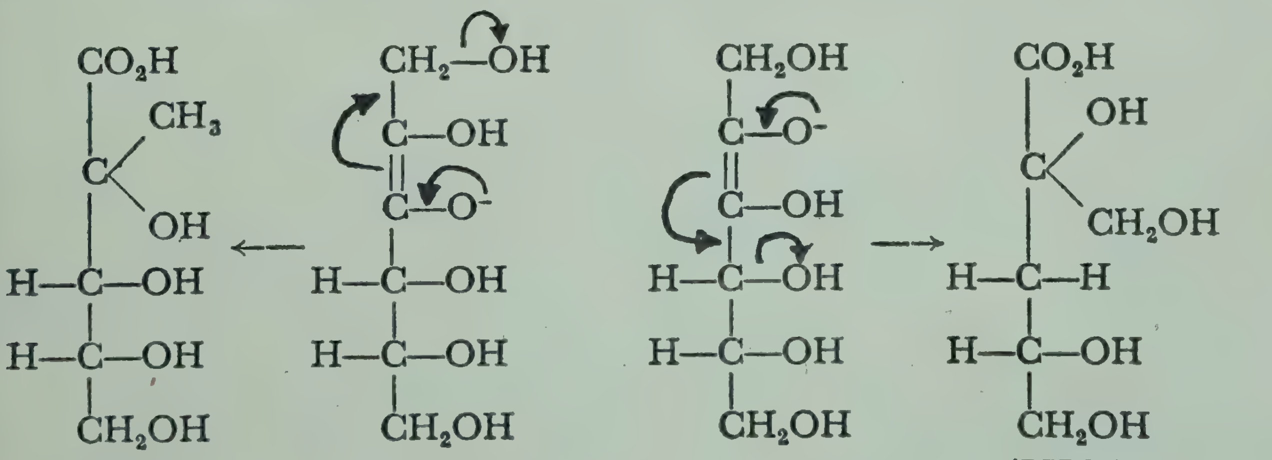
Isbell[11] in 1944 postulated a mechanism based on consecutive electron-displacement reactions to explain the formation of saccharinic acids by the action of alkali upon the sugars. The theory is in effect a more modern version of an earlier one proposed by Nef.[12] The first stage involves the formation and ionization of an enediol, followed by a β-elimination of a hydroxyl group. (This type of elimination is quite well known in the β-hydroxyaldehyde series; for instance the conversion of aldol into crotonaldehyde by alkali.) The resulting enediol then undergoes rearrangement to an α-dicarbonyl intermediate which, by a benzilic acid rearrangement, gives the saccharinic acid.



$$\begin{array}{c} C_6H_5{-}CO \\ | \\ C_6H_5{-}CO \end{array} \longrightarrow \begin{array}{c} (C_6H_5)_2C{-}OH \\ | \\ COOH \end{array}$$

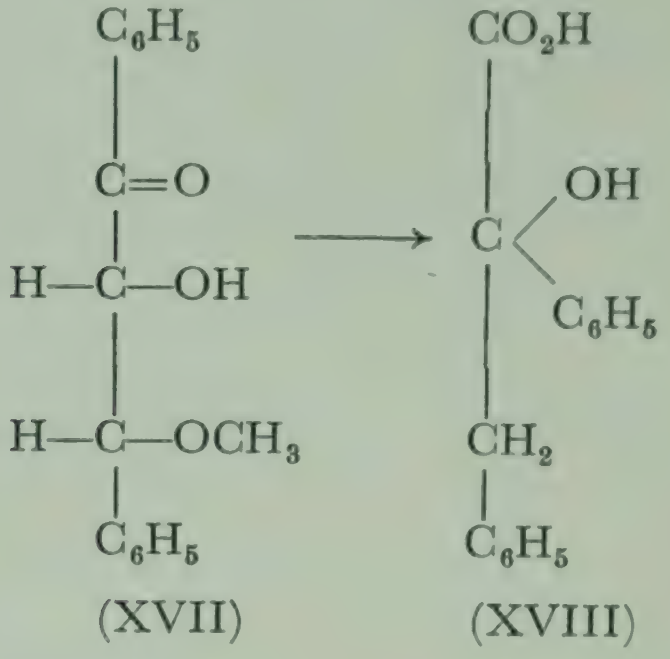
Below is outlined the mechanism for the production of *meta*-saccharinic acid from glucose. The ion of the 2 : 3 enediol will give rise to either saccharinic acid (XV) or *iso*saccharinic acid (XVI)





(XV) (XVI)

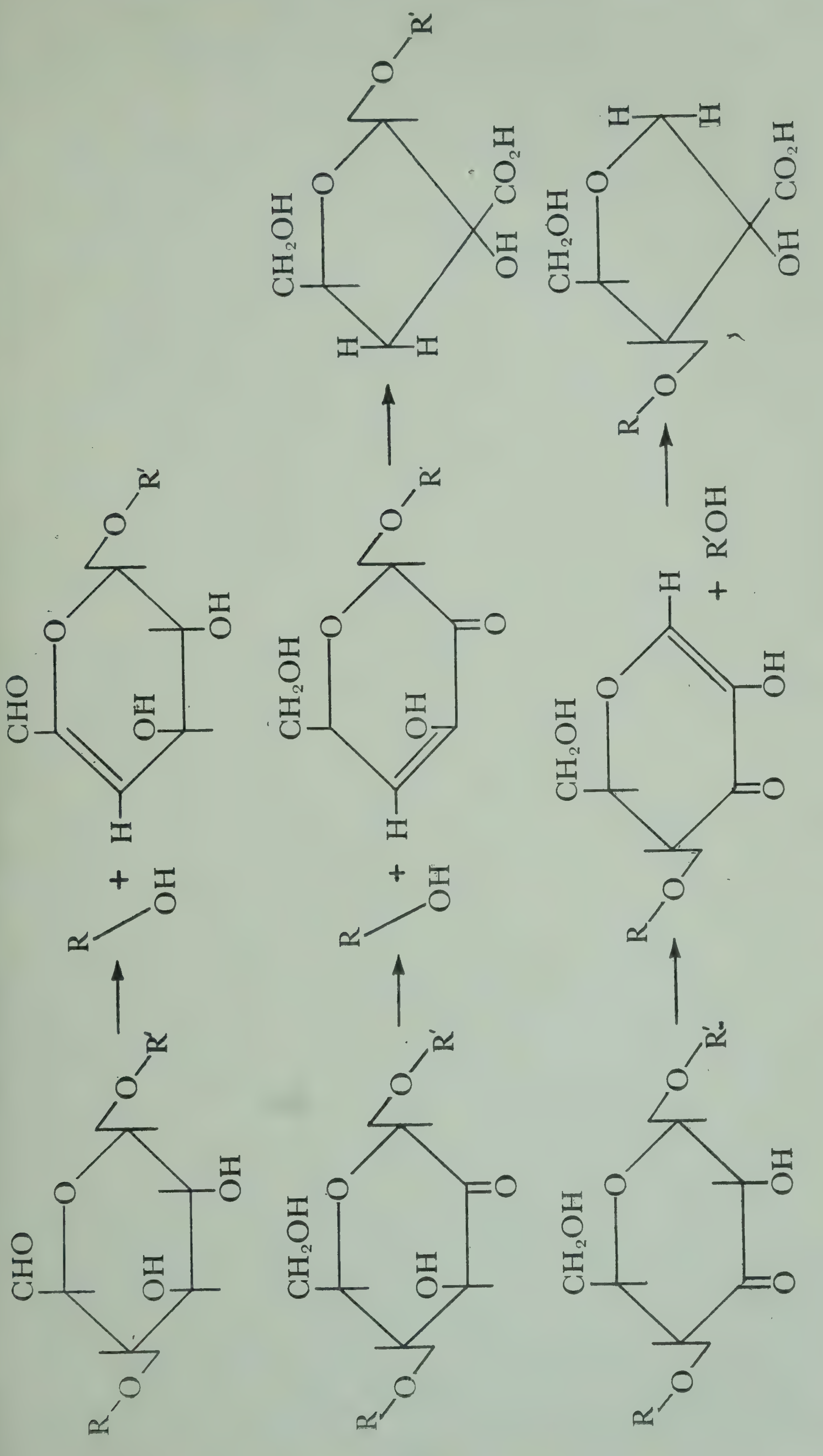
according to whether the ion is located at $C_{(3)}$ or $C_{(2)}$. This type of mechanism readily explains the earlier observation of Nicolet[13] that treatment of 2-hydroxy-3-methoxy-3-phenyl-propiophenone (XVII) with alkali gives diphenyllactic acid (XVIII).



The mechanism involves the splitting out of a hydroxyl ion into a solution containing hydroxyl ions, and therefore by mass action effects, it would be expected that this type of reaction will be slow and will occur only to a limited extent. Indeed, this is so in the case of the alkaline degradation of glucose in which the saccharinic acids produced are about 50 per cent of the total acids. Later it will be shown that if an alkoxy group is split out instead of a hydroxyl group, the β-alkoxycarbonyl elimination reaction will become essentially the only reaction, and the rate of reaction will be greatly increased.

This mechanism has been applied to oxidized celluloses by Haskins and Hogsed.[14] An examination of the basic repeating unit of cellulose will show that should any one of the three hydroxyl groups be oxidized, a β-alkoxycarbonyl system will be produced which, according to the above theory would be labile to alkali and be responsible for chain scission.

Alkaline degradation of a glucose unit oxidized at either the $C_{(3)}$ or $C_{(2)}$ hydroxyl group will lead to the formation of an acidic product attached to the reducing or non-reducing end, respectively, of the cleaved cellulose molecule. In contrast, attack of alkali upon a glucose unit oxidized at the primary hydroxyl group will lead to chain scission but not to the formation of an acidic product because of the absence of a free hydroxyl group in a position α to the aldehyde group (see page 121).



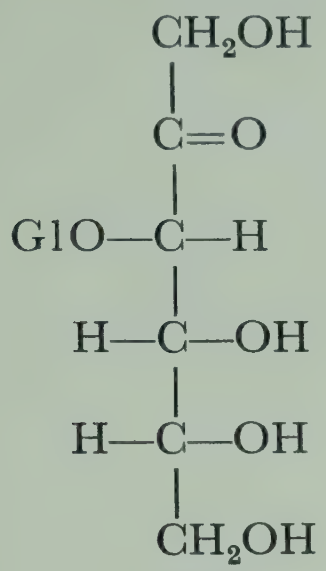
ALKALINE DEGRADATION OF MODEL COMPOUNDS OF CELLULOSE AND OXIDIZED CELLULOSE

Except in the case of a few reagents (e.g. periodic acid, lead tetraacetate and nitrogen dioxide) the action of oxidants upon a polysaccharide is far from specific and a very complex oxidized polysaccharide is produced. The action of various oxidants under various conditions of pH values upon cellulose has been followed by determining the number of carboxylic, aldehydic, and ketonic groups produced.[15] The methods of determining these groups are far from perfect and, consequently, the results obtained must be considered with caution. However, oxidation under acidic conditions usually results in the production of aldehydic or ketonic groups, whereas oxidation under alkaline conditions produces mainly carboxylic acids. This is to be expected from any of the three proposed mechanisms since they all postulate the eventual production of acidic products from the action of alkali upon a carbonyl oxycellulose.

In order to determine the mechanism responsible for alkaline degradation of chemically modified celluloses, Corbett, Kenner, and Richards[16] have studied the action of alkali upon simple sugar derivatives (model compounds) which resemble the oxycelluloses. The main features of any alkali labile oxycellulose are two in number, namely a carbonyl group and a glycosidic link. The simplest type of compound with these requirements are the disaccharides and, consequently, the action of lime-water upon these compounds has been studied. The work was extended to cover alkyl ethers of monosaccharides since they react in a similar manner, and are often more readily obtainable than the corresponding disaccharides. It will be realized that these model compounds could undergo alkaline degradation by either the Evans' or β-alkoxycarbonyl elimination mechanisms, but that the mechanism postulated by Pacsu could not possibly operate.

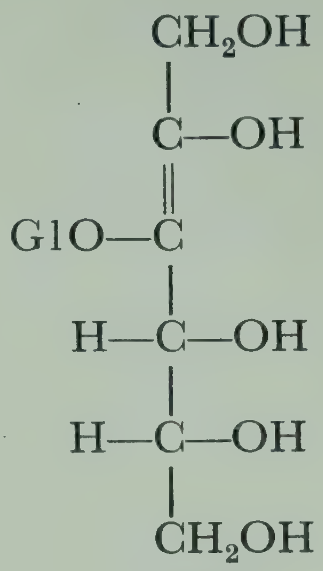
3-O-*Substituted Monosaccharides*

Isbell[17] observed that the optical rotation of an alkaline solution of turanose (3-*O*-α-D-glucosyl-D-fructose, XIX) decreased whereas the reducing power towards an alkaline copper reagent increased to a maximum and then decreased. These changes could not be ascribed to a simple de Bruyn–van Ekenstein transformation, and were consequently attributed to turanose being converted by the alkali into the enediol (XX) which, having the necessary structure for alkaline hydrolysis as required by Evans' theory, underwent hydrolysis to glucose and fructose.



$$\begin{array}{rcl} & \mathrm{CH_2OH} & \\ & | & \\ & \mathrm{C{=}O} & \\ & | & \\ \mathrm{GlO{-}} & \mathrm{C} & \mathrm{{-}H} \\ & | & \\ \mathrm{H{-}} & \mathrm{C} & \mathrm{{-}OH} \\ & | & \\ \mathrm{H{-}} & \mathrm{C} & \mathrm{{-}OH} \\ & | & \\ & \mathrm{CH_2OH} & \end{array}$$

(XIX)



$$\begin{array}{rcl} & \mathrm{CH_2OH} & \\ & | & \\ & \mathrm{C} & \mathrm{{-}OH} \\ & \| & \\ \mathrm{GlO{-}} & \mathrm{C} & \\ & | & \\ \mathrm{H{-}} & \mathrm{C} & \mathrm{{-}OH} \\ & | & \\ \mathrm{H{-}} & \mathrm{C} & \mathrm{{-}OH} \\ & | & \\ & \mathrm{CH_2OH} & \end{array}$$

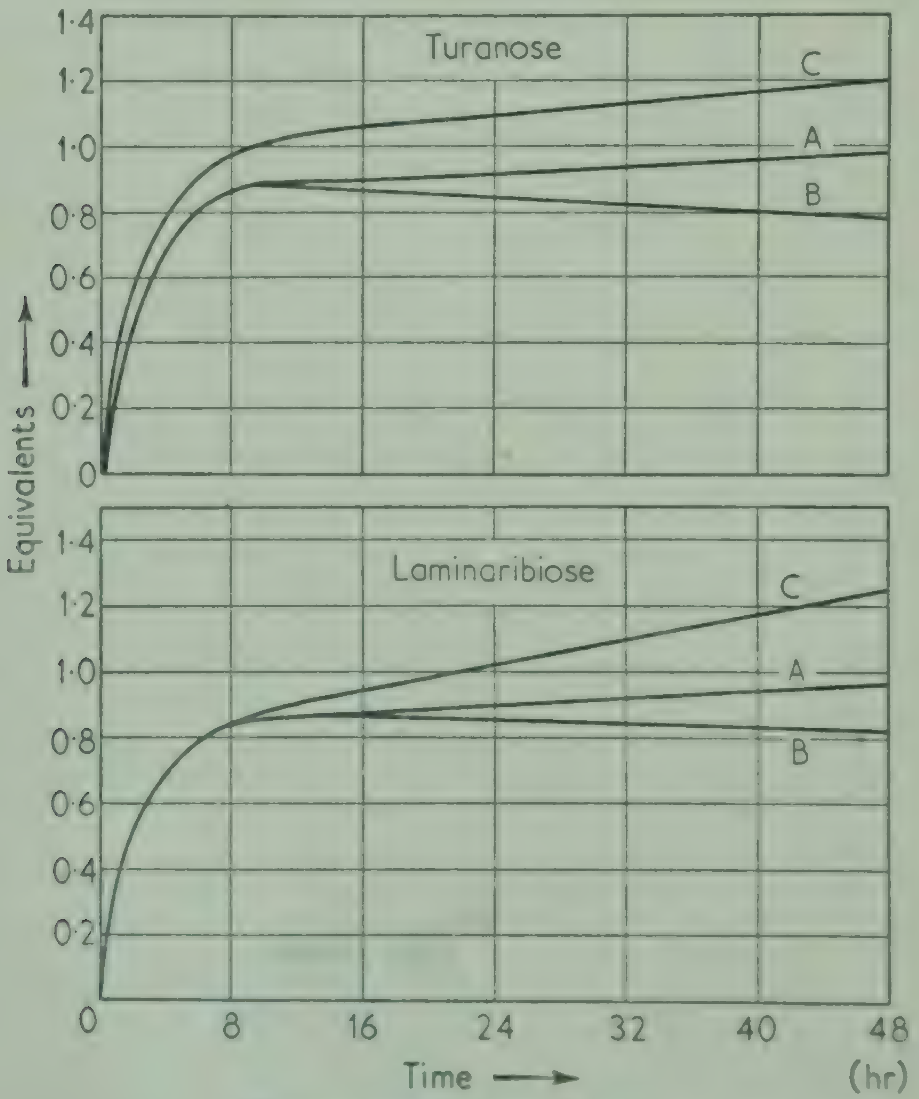
(XX)

Re-examination[16a] of the action of alkali upon turanose confirmed that the disaccharide is rapidly degraded in alkaline solutions. Analysis of the solution, however, showed that the disaccharide was not hydrolysed to a mixture of glucose and fructose, but that it was converted essentially into 1 mole of acid and 1 mole of glucose (Figure 1). Chromatographic analysis of the reaction mixture indicated that glucose was the only monosaccharide produced in the early stages, and fructose (and psicose) was only detected in the later stages, presumably by isomerization of the glucose. The acid was isolated as the brucine salt and was shown to be essentially *meta*saccharinic acid.

These results show that the reaction follows the β-alkoxycarbonyl elimination mechanism. At first it may be difficult to see how the latter reaction can occur when the glucosidic group is in a position α to the carbonyl group. However, study of the ions will show how it occurs. Turanose can ionize in two ways. It can form the ion (XXI) which may be responsible for a reversed aldol condensation to give glycerose and glucosyl-dihydroxyacetone, both of which will ultimately be converted into lactic acid, and for the less favourable β-elimination of the $C_{(4)}$ hydroxyl group which will not give rise to acidic products. The disaccharide can also ionize to furnish the ion (XXII) which would be convertible into the ion (XXIII). The interconversion of the two ions would be rapid, and the structure of the ions may perhaps be better represented by the resonating structure (XXIV). The fact that *meta*saccharinic acid is the major acidic product and only small amounts of lactic acid are produced indicates that the ion (XXII) predominates over ion

(XXI). The production of lactic acid explains the small excess acid observed during the initial stages of the reaction (Figure 1).

As anticipated, laminaribiose (3-*O*-β-D-glucosyl-D-glucose) (XXV is also readily degraded by alkali to yield essentially an equimol cular mixture of glucose and *meta*saccharinic acid (Figure 1). I this particular case the disaccharide has the required β-alkoxy carbonyl structure without modification, and therefore its io (XXIII) would be expected to be degraded rapidly. This is s but no more rapidly than the ion of turanose. These apparentl similar rates of degradation provide evidence that the intercon version of the ions (XXII) and (XXIII) is fast, or even that the exist as the resonating form (XXIV). The rate controlling reactio is the elimination of the glucosyl group to furnish the enol of th dicarbonyl compound (XXVI). Since laminaribiose is an aldos derivative, the possibility of the formation of the ion (XXI) i remote and, therefore, it may be predicted that no lactic acid woul



(Corbett and Kenner. *J. Chem. Soc.* 3274 (1954))

A: Disaccharide decomposed;
B: Monosaccharides produced;
C: Total acids produced

Figure 1. The degradation of turanose and laminaribiose by saturated lime-water at 25°

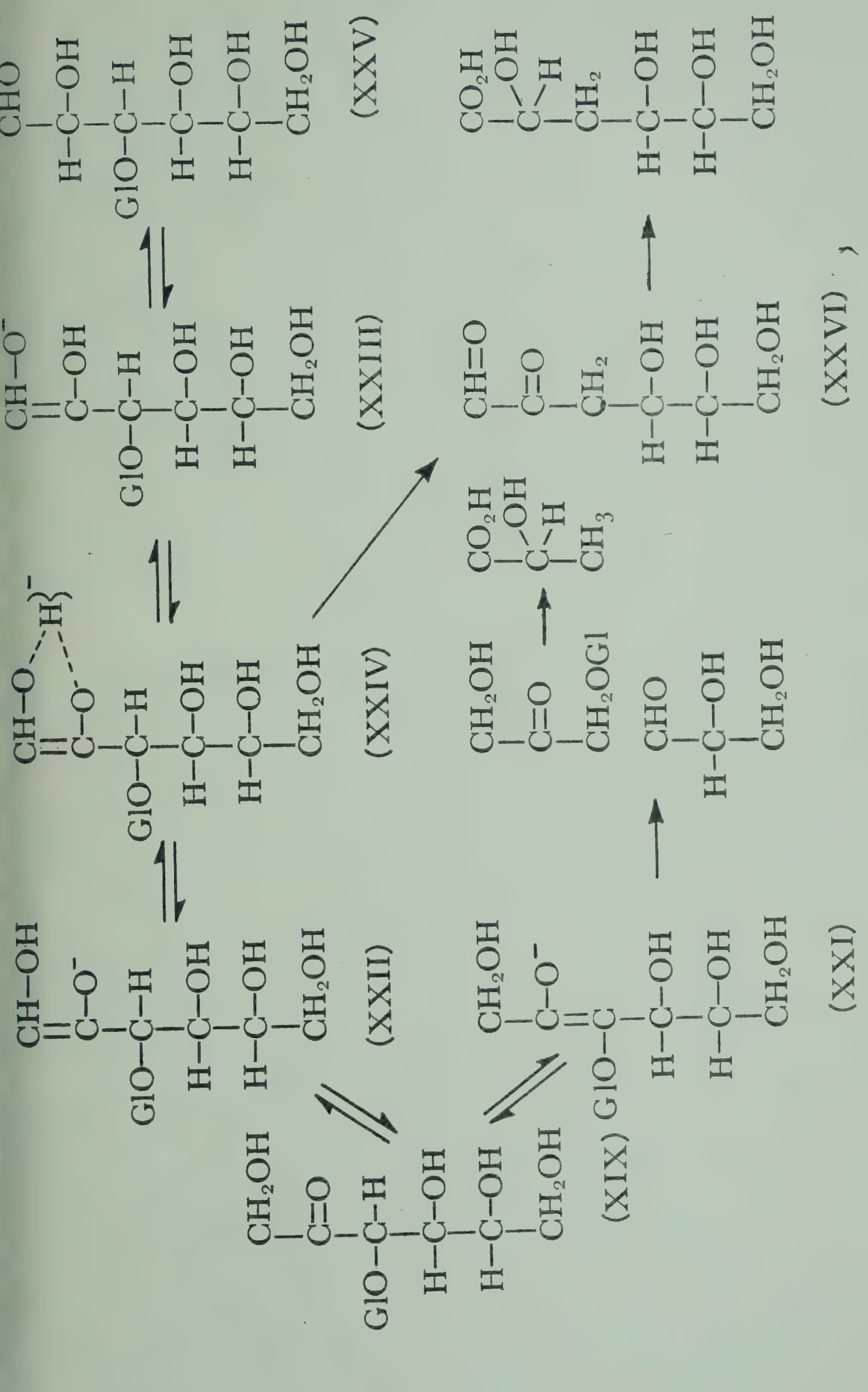

(XIX)
(XXII)
(XXIV)
(XXIII)
(XXV)
(XXI)
(XXVI)

Figure 2. Structure representing degradation of laminaribose by alkali

be produced from the reducing glucose unit. This was found to the case, for in the early stages of the degradation (i.e. before t glucose began to be degraded) only 1 equivalent of acid w produced per mole of disaccharide decomposed (Figure 1).

Several other 3-*O*-substituted derivatives of monosaccharides ha been studied, and are listed together with their degradation produ in Table 1. In all cases it was shown that a β-alkoxycarbo elimination mechanism was primarily responsible for the observ degradation. Particular mention should be made of the 3-meth ethers of fructose and glucose[16b] which behaved in a manner ve similar to turanose and laminaribiose except that they degraded mo slowly. The influence of the mass action effect has been conclusive established by the fact that the rate of degradation of 3-*O*-methyl-glucose was found to be much slower in methanolic solutions

Table 1: Main products isolated from the degradation of 3-*O*-substituted monosaccharides by alkali

Derivative	Reference	Main products isolate
3-*O*-Methyl-D-glucose ...	*J. Chem. Soc.* 278 (1954)	D-Gluco*meta*saccharini acid (3-deoxy-D-hexon acid)
D-Glucose 3-phosphate ...	*Ber.* **90**, 936 (1957)	
3 : 6-Anhydro-D-glucose	*J. Chem. Soc.* 927 (1957)	
Laminaribiose	*J. Chem. Soc.* 3274 (1954)	
3-*O*-Methyl-D-fructose ...	*J. Chem. Soc.* 278 (1954)	
Turanose...	*J. Chem. Soc.* 3274 (1954)	
3 : 6-Di-*O*-methyl-D-glucose	*J. Chem. Soc.* 2916 (1956)	6-*O*-Methyl-D-gluco*meta* saccharinic acid
2 : 3-5 : 6-Di-*O*-*iso*propylidene-D-mannose	*J. Chem. Soc.* 1709 (1955)	5 : 6-*O*-*iso*Propylidene-D-gluco*meta*saccharini acids
3-*O*-Methyl-D-xylose ...	*J. Chem. Soc.* 11 (1957)	C_5-D-*Meta*saccharinic acid (3-Deoxy-D-pentonic acid)
D-Ribose 3-phosphate ...	*Ber.* **90**, 936 (1957)	

Table 2: Apparent first-order velocity constants for 3-*O*-methyl-D-glucose wi various sodium alkoxides* at 25°

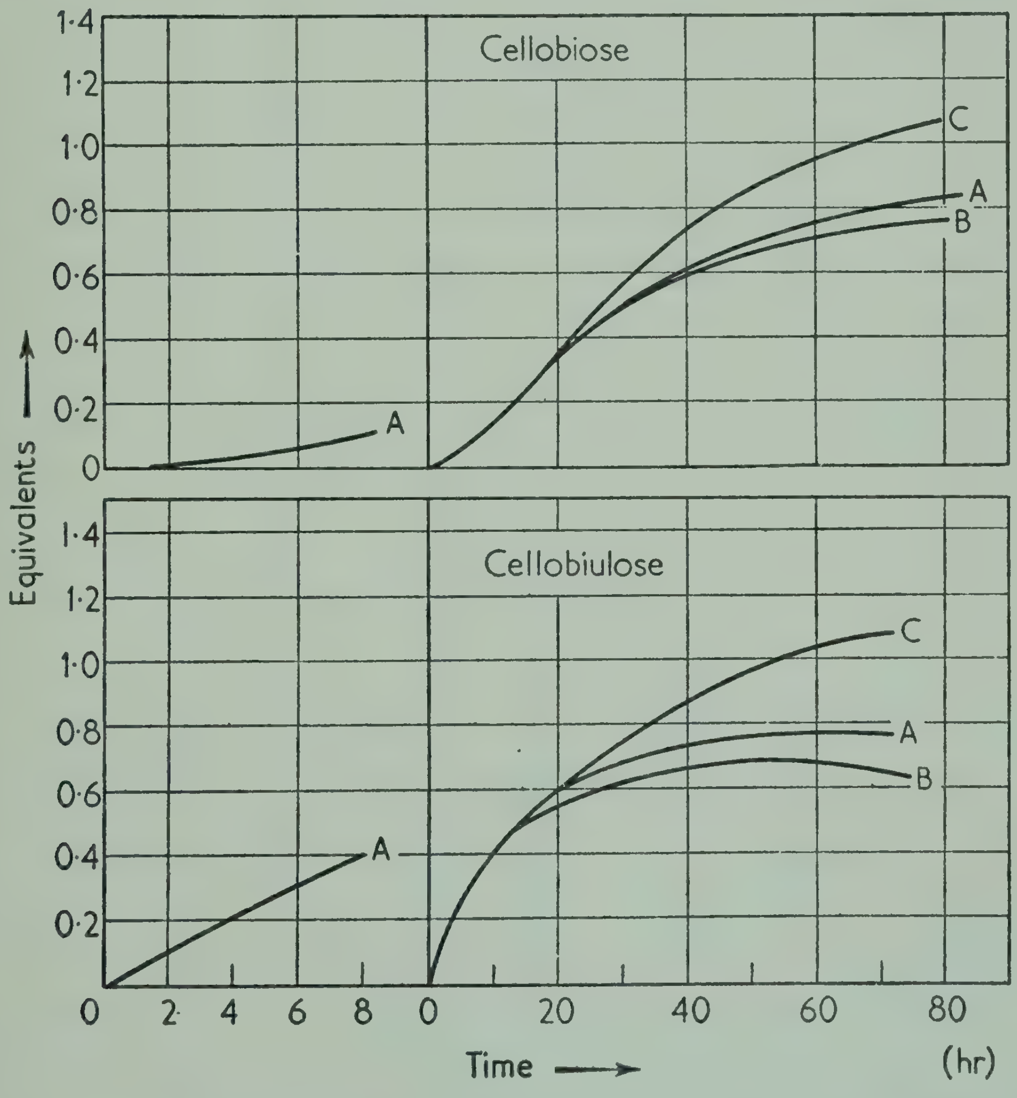
0·1N Alkali	Apparent velocity constant ($\times 10^2$ hr^{-1})
NaOH—H_2O ...	4·0
NaOMe—MeOH ...	0·7
NaOEt—EtOH ...	6·3

* J. Kenner and G. N. Richards. *J. Chem. Soc.* 3019 (1957)

sodium methoxide than in aqueous solutions of sodium hydroxide or in ethanolic solutions of sodium ethoxide (Table 2).

4-O-Substituted Monosaccharides

Solutions of 4-*O*-substituted derivatives of aldoses are initially stable towards alkali (Figure 3, top left graph). However, degradation does begin to occur after a time lag of several hours, and 1 mole of disaccharide decomposes to 1 mole of monosaccharide and 1 equivalent of acid.[16c] With 4-*O*-substituted derivatives of the ketoses there is no observed time-lag. These facts can only be satisfactorily explained by a β-alkoxycarbonyl elimination reaction. The ketose derivatives (XXVIII) contain the requisite β-alkoxycarbonyl system, and therefore are degraded through their ion (XXIX) (Figure 3). The aldose derivatives (XXVII) are initially stable towards alkali because (a) they do not contain a β-alkoxycarbonyl system, and (b) the presence of the substituent on the $C_{(4)}$

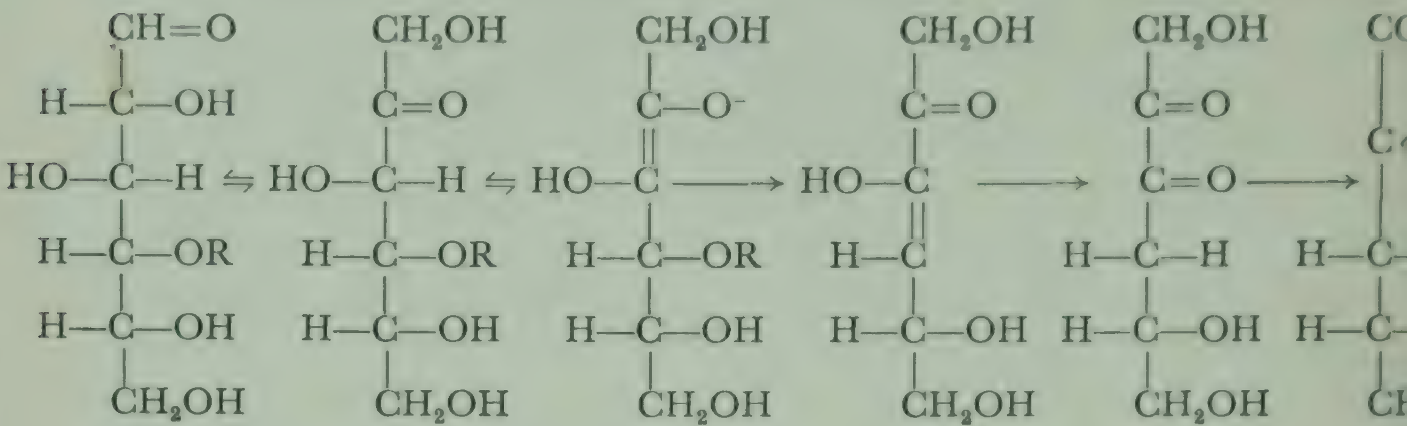


(Corbett and Kenner. *J. Chem. Soc.* 1431 (1955))

A: Disaccharide decomposed;
B: Monosaccharide produced;
C: Total acids produced

Figure 3. The degradation of cellobiose and cellobiulose by saturated lime-water at 25°

hydroxyl group prevents the occurrence of a reversed aldol conden tion reaction which would ultimately give rise to lactic acid. In presence of alkali, however, the aldose undergoes a de Bruyn– Ekenstein transformation to the ketose which is then capable degradation. A time-lag in the degradation would be expec during which period the concentration of ketose would be built



(XXVII) (XXVIII) (XXIX) (X

Further evidence for the *β*-alkoxycarbonyl elimination has be provided by the isolation of the major proportion of the acid products and their identification as *iso*saccharinic acid (XXX).

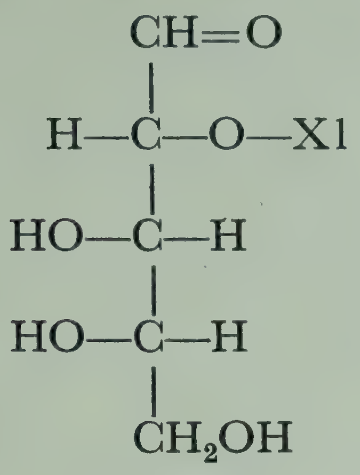
Numerous 4-*O*-substituted monosaccharides have been degrad by alkali and their degradation products identified. These are list in Table 3.

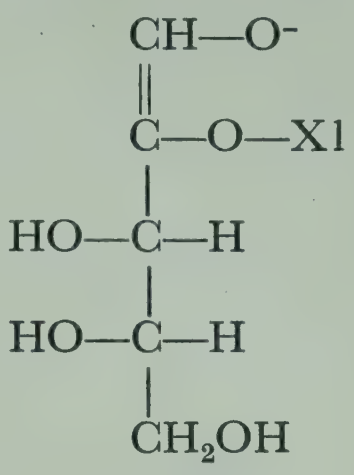
Table 3: Main products isolated from the degradation of 4-*O*-substituted monosaccharides by alkali

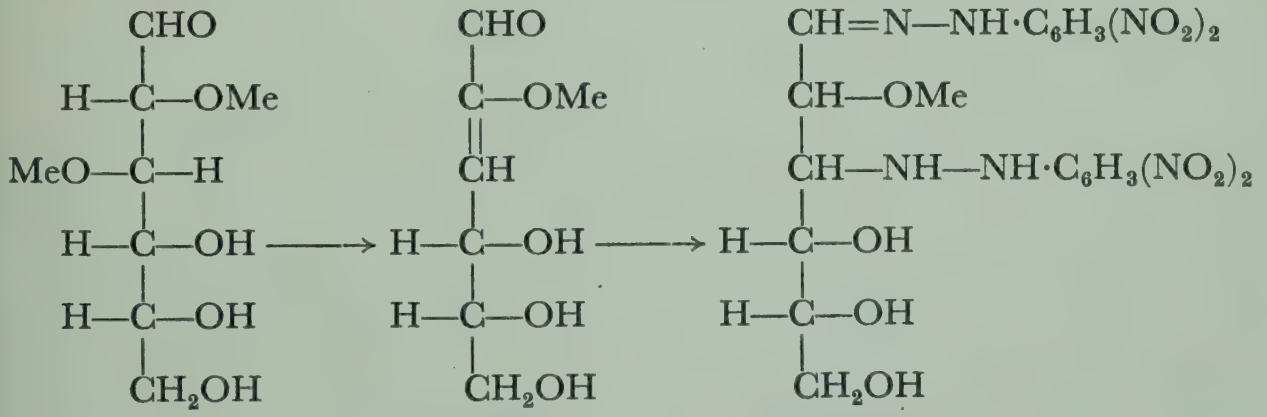
Derivative	Reference (*J. Chem. Soc.*)	Main product isolated
4-*O*-Methyl-D-glucose 4-*O*-Methyl-D-fructose ...	1810 (1955)	*iso*Saccharinic acid (3-Deoxy-2-*C*-hydroxy methyl-pentonic aci
Maltose Maltulose	1789 (1954)	
Lactose	2245 (1953)	
Lactulose	1789 (1954)	
Cellobiose Cellobiulose	1431 (1955)	
4 : 6-*O*-Benzylidene-D-glucose	1709 (1955)	
4 : 6-Di-*O*-methyl-D-glucose	2916 (1956)	5-*O*-Methyl-*iso*sacchar-inic acid
Xylobiose	4807 (1956)	$C_{(5)}$-*iso*Saccharinic aci (2 : 4-Dihydroxy-2-hydroxy-methylbut acid)

2-Mono- and 2 : 3-Di-O-substituted Monosaccharides

2-*O*-D-Xylopyranosyl-L-arabinose (XXXI) has been shown[18] to be stable toward alkali at 25° even though the ion (XXXII) has the structure which, according to Evans' theory, is responsible for alkaline hydrolysis. At higher temperatures (100°), the disaccharide is degraded into acidic products, but the nature of the products and the mechanism of degradation have not been determined. According to the Isbell theory, elimination of the $C_{(3)}$ hydroxyl group would be expected to occur, although to a limited extent because of the mass action effect, but this would not result in acidic products since the $C_{(2)}$ hydroxyl group is substituted. The fact that a β-alkoxycarbonyl elimination can occur although the α-hydroxyl group is substituted is illustrated[16d] by 2 : 3-di-*O*-methyl-D-glucose (XXXIII). In the presence of lime-water, this disubstituted derivative eliminates 1 mole of methanol to give the enol (XXXIV) which was isolated as the *bis*-2 : 4-dinitrophenylhydrazone (XXXV). The significant points of these cases are: (a), β-elimination can occur even when the α-hydroxyl group is substituted; and (b), a substituent at the α-position is relatively stable towards alkali. These findings are of importance when the oxidized celluloses are considered.


(XXXI)


(XXXII)


(XXXIII) (XXXIV) (XXXV)

Oligo- and Polysaccharides

A study of the structure of an oligosaccharide will indicate that alkaline degradation by a β-alkoxycarbonyl elimination should apply not only to simple monosaccharide derivatives but also to the more complex carbohydrates in which the reducing terminal sugar unit will react in a manner similar to that of the reducing unit of a disaccharide, and the remaining units will function as the substituent controlling the direction of degradation. If the reducing terminal unit of an oligo- or polysaccharide exists as, or can be transformed by a de Bruyn–van Ekenstein transformation into a β-alkoxycarbonyl structure, the unit will be cleaved off as the enol of a dicarbonyl which will be converted into a saccharinic acid by further reaction with alkali. The structure of the acid will be controlled by the position of attachment of the residual glucosyl units. When the reducing unit is cleaved off, the next glucose unit will be exposed to alkali and will be capable of undergoing alkaline degradation. Thus, a stepwise alkaline degradation occurs from the reducing end of the molecule, and with the more simple oligosaccharides will proceed until the whole molecule has been degraded. Such a reaction was observed with cellotetraose[16c] and the stages in its degradation, as observed by paper chromatography, may be represented by the following scheme.

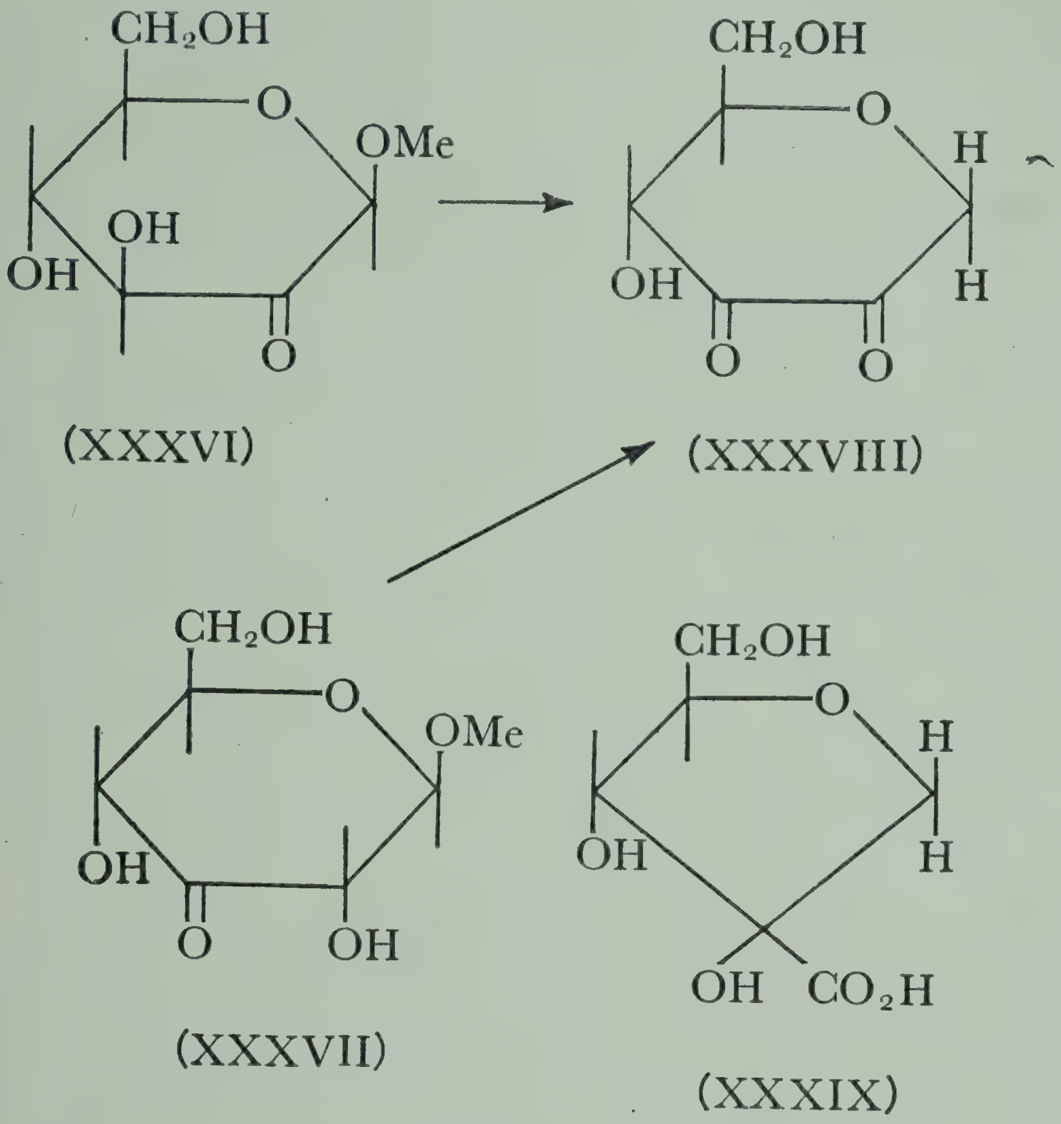
$$\begin{tikzcd} & \text{G—G—G—G} \arrow[r] & \text{G—G—G—Fru} \arrow[r] & \text{G—G—G} + i\text{Sac} \arrow[d] \\ \text{G} + i\text{Sac} & \text{G—Fru} \arrow[l] & \text{G—G} + i\text{Sac} \arrow[l] & \text{G—G—Fru} \arrow[l] \end{tikzcd}$$

(G = glucosyl unit; Fru = fructose unit; *i*Sac = *iso*saccharinic acid.)

A similar reaction occurs with laminarin, a polysaccharide which consists essentially of D-glucopyranosyl units linked by β-1-3 links. In this particular case it is impossible to follow the course of the reaction, but the anticipated acid, *meta*saccharinic acid, may be isolated in good yield.

Carbonyl Derivatives of Monosaccharides

The oxidation of methyl β-D-glucoside by chromic acid produces various carbonyl derivatives which may be separated by chromatography.[19] These new derivatives are perfect model compounds for oxycelluloses, and no doubt in the near future their reaction towards alkali will be studied in detail. Preliminary results of such a study have been announced[20] for methyl 2- (and 3-) didehydro-β-D-glucoside (XXXVI and XXXVII, respectively). As anticipated, both carbonyl compounds are readily degraded by alkali to the same dicarbonyl compound which, from an examination of its reduction products, would appear to have the structure (XXXVIII). On further treatment with alkali the dicarbonyl compound is converted into an acidic product which, from the previously described similar cases, would be expected to have the structure (XXXIX).



(XXXVI)

(XXXVIII)

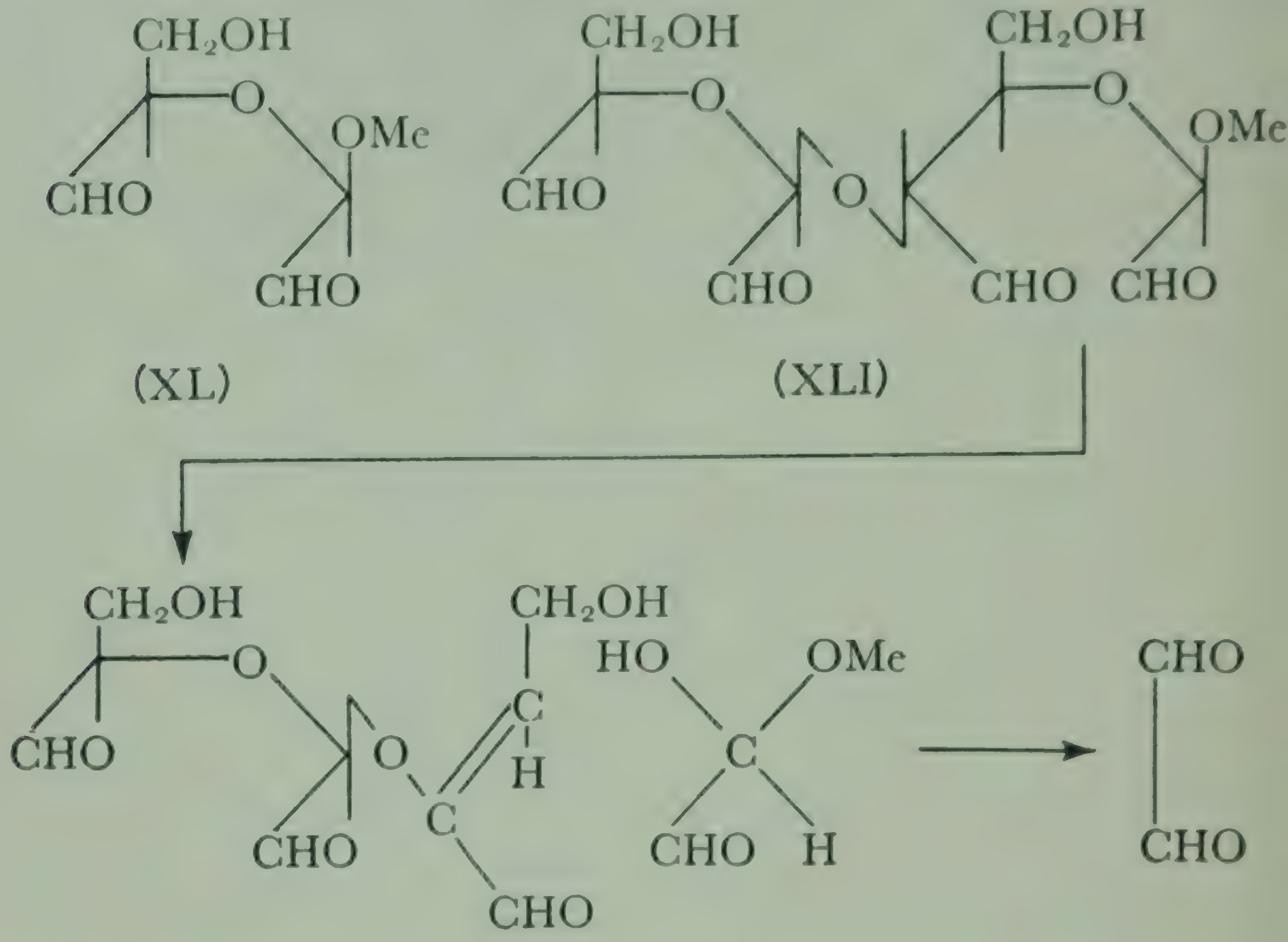
(XXXVII)

(XXXIX)

Periodate-oxidized Glycosides of Mono- and Disaccharides

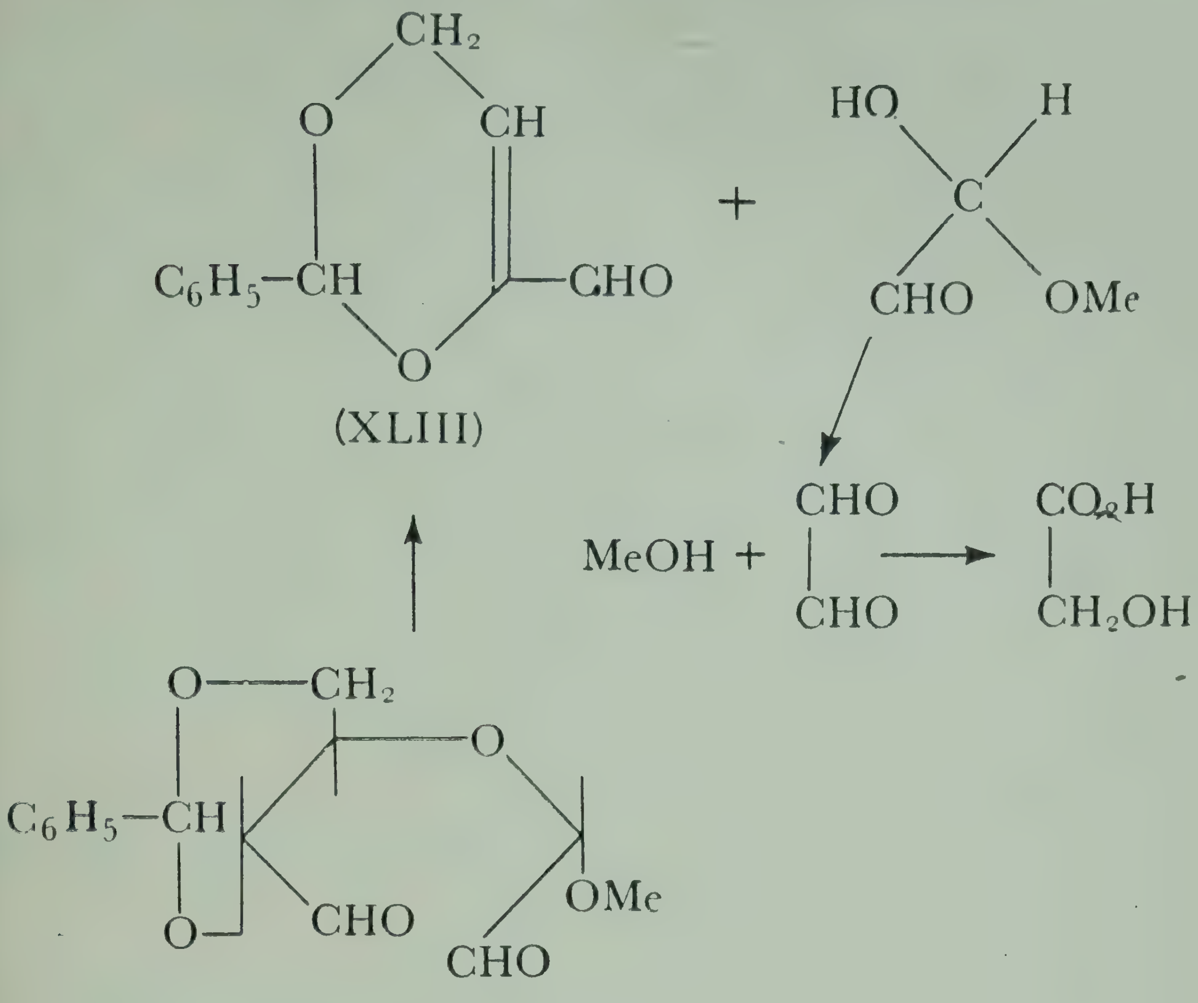
Head[21] has studied the action of various alkalis upon the periodate oxidation products of methyl β-D-glucoside (XL) and of β-cellobioside (XLI). In the presence of sodium carbonate or sodium hydroxide the dialdehyde slowly produced acidic products accompanied by a reduction in the number of carbonyl groups. After 24 hr in sodium hydroxide (0·1N), the number of carbonyl groups degraded was 2·8 times the number of carboxylic acid groups produced. These results tend to indicate that the major reaction was a Cannizzaro reaction of the type previously observed by Fry, Wilson, and Hudson.[9] In contrast, the tetra-aldehyde (XLI) was shown to be quite labile to alkali and glyoxal was isolated.

Examination of the structures of the tetra- and dialdehyd reveals that the former contains an α- and a β-alkoxycarbon system whereas the latter contains only an α-alkoxycarbonyl syste The mechanisms of Evans and of Pacsu predict that the dialdehyd and tetra-aldehyde would be equally labile towards alkali, wherea


(XL) (XLI)

the β-alkoxycarbonyl elimination mechanism accords with th observed fact that the dialdehyde is more stable than the tetr aldehyde. Further, the production of glyoxal cannot be explaine by Pacsu's theory, whereas that of Evans would predict its produ tion from both the tetra- and dialdehyde. The β-alkoxycarbon elimination is alone in predicting that the tetra-aldehyde on should give glyoxal.

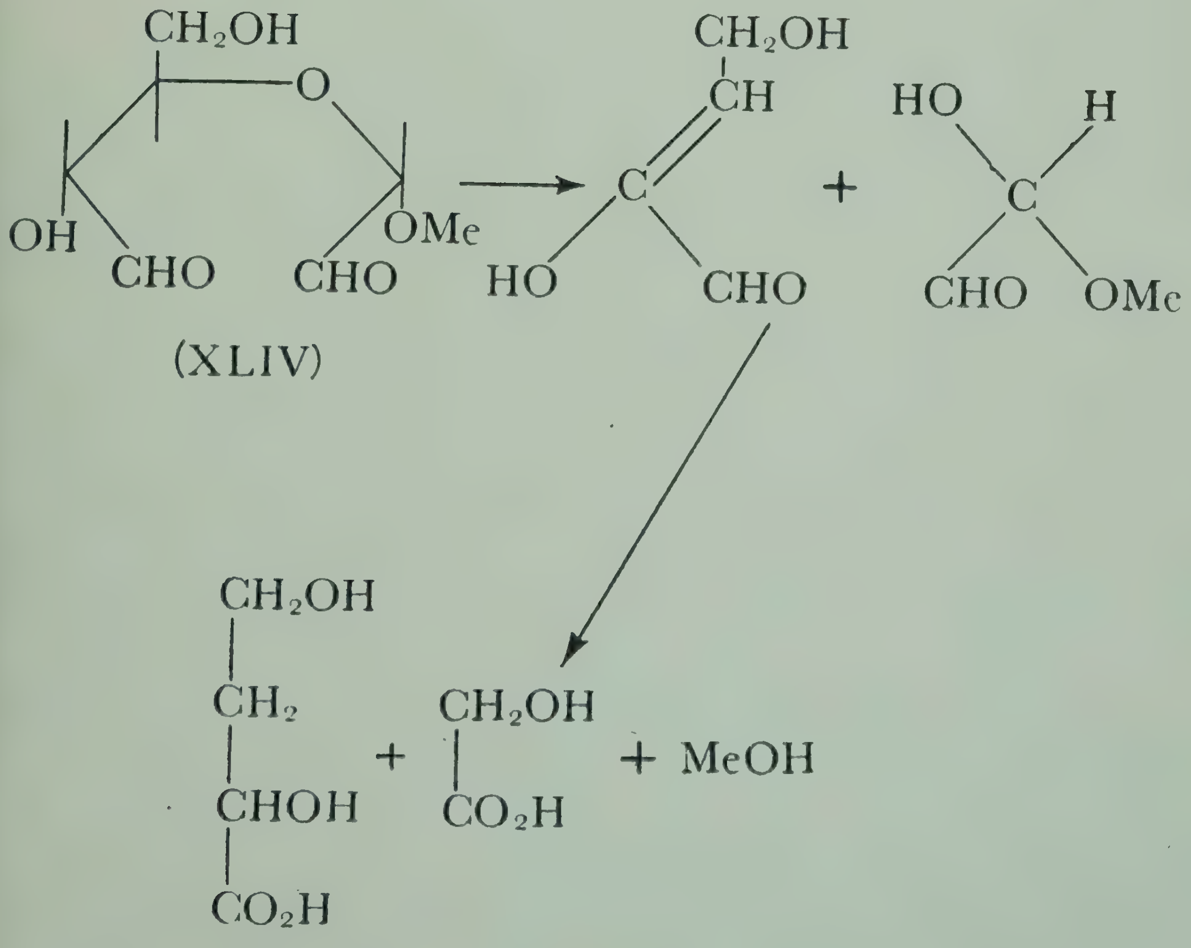
Further studies on similar lines have been made by O'Meara a Richards.[22] These authors found that the periodate oxidati product of methyl 4 : 6-*O*-benzylidene-α-D-glucoside (XLII) react with lime-water. Besides the products arising from a Cannizza reaction there were also isolated, in approximately equal quantiti glycollic acid and a compound which behaved as 4-formyl-phenyl-2*H*-6*H*-1 : 3-dioxin (XLIII). The formation of glycol acid can be explained by Pacsu's theory which predicts its dir formation, or by the theory of Evans or the β-alkoxycarbo elimination, since the glyoxal predicted in these latter theor



(XLIII)

(XLII)

CH2
O
CH
C6H5—CH
CHO
O
+
HO
H
C
CHO
OMe
MeOH +
CHO
CHO
CO2H
CH2OH
O
CH2
C6H5—CH
O
CHO
CHO
OMe



(XLIV)

(XLV)

CH2OH
O
OH
CHO
CHO
OMe
CH2OH
CH
C
HO
CHO
+
HO
H
C
CHO
OMe
CH2OH
CH2
CHOH
CO2H
+
CH2OH
CO2H
+ MeOH

would undergo a Cannizzaro reaction to give glycollic acid. Form
tion of the dioxin derivative can, however, only be satisfactori
explained by Isbell's mechanism.

Removal of the benzylidene group by acid hydrolysis of th
dialdehyde (XLII) gives the dialdehyde (XLIV) which is degrade
by alkali to approximately equal amounts of α : γ-dihydroxybutyr
acid (XLV) and glycollic acid. Again, these results can only b
satisfactorily explained by the β-alkoxycarbonyl elimination mech
anism. The other two theories predict the formation of D-erythros
and glycollic acid. While it is possible that alkaline solutions
D-erythrose may give α : γ-dihydroxybutyric acid, it is not expecte
to be the sole acid as would be necessary to explain the observe
results. Indeed, it has been shown[23] that D-erythrose is not converte
by alkali into appreciable quantities of α : γ-dihydroxybutyri
acid.

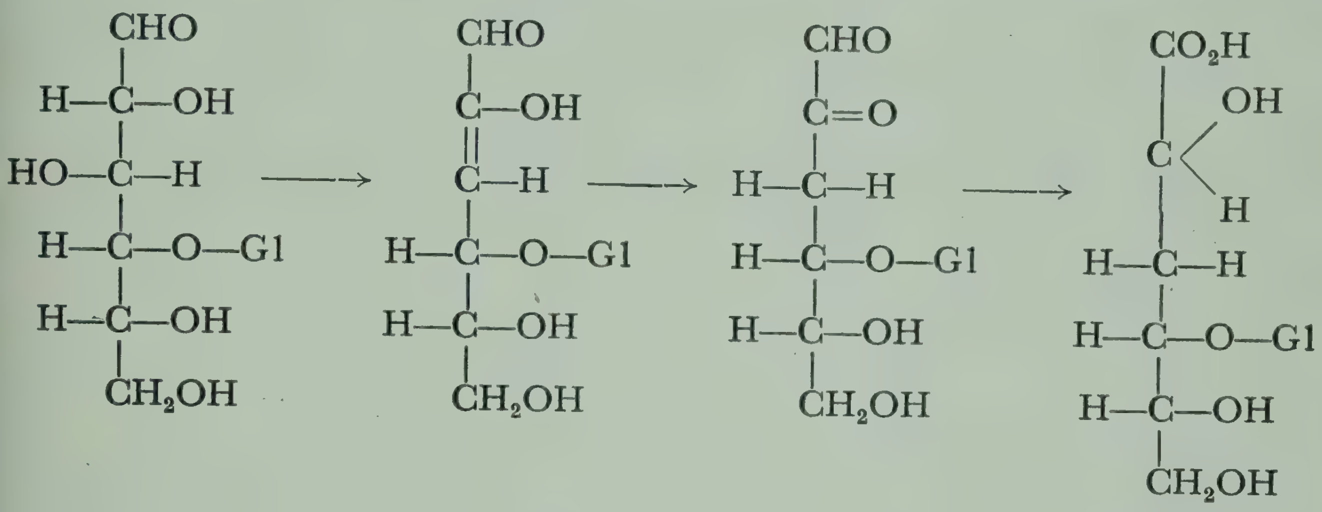
THE ALKALINE DEGRADATION OF CHEMICALLY MODIFIED CELLULOS

Hydrocellulose and Cellulose

Cellulose is only very slowly attacked by alkali because of the ver
small number of carbonyl groups compared with the number
glucosidic linkages. Hydrocellulose (partially hydrolysed cellulose
has virtually the same chemical structure as cellulose itself, an
therefore several groups of workers have used this material for thei
studies. Degradation will be increased owing to the increase
number of carbonyl groups, but the nature of the products will b
the same. An examination of the sodium hydroxide degradatio
products of hydrocellulose has shown them to be extremely com
plex.[24] Identification of the major acid component as *iso*sacchari
acid (XXX) indicates that the major reaction is a stepwise alkali
degradation from the reducing end (cf. page 122). However, th
identification of some of the other acids as formic, dihydroxybutyri
acetic, lactic, and glycollic, indicates that reactions other than
β-alkoxycarbonyl elimination may be occurring. Some of the aci
may possibly arise from the action of alkali (sodium hydroxide
these particular cases) upon the dicarbonyl intermediate resulti
from a β-alkoxycarbonyl elimination. Most of the model compou
work previously described employed calcium hydroxide, which
thought to catalyse the benzilic acid rearrangement to sacchari
acids. With sodium hydroxide, the alkali may not be so specific a
smaller acids may be produced from the dicarbonyl intermediate

The alkaline degradation of hydrocellulose never goes to compl
tion but, ultimately, the polysaccharide becomes stable towar

alkali. It has been observed[26] that during the degradation the cellulose becomes acidic, and it was concluded that the stabilization is due to the production of these acidic groups. The nature of this stabilizing or ‘ stopping ’ reaction has been investigated by Machell and Richards,[27] who submitted an alkali-stabilized hydrocellulose to acid hydrolysis, and isolated the resulting acidic products. The major proportion of these products was identified as D-*meta*saccharinic acid. The interpretation of this finding is that whilst most of the reducing end units in the cellulose molecule undergo a β-alkoxycarbonyl elimination at the $C_{(4)}$ atom through the fructose intermediate, a few undergo a β-hydroxycarbonyl elimination of the $C_{(3)}$ hydroxyl group through the aldose form to give a substituted enol (XLVI). The latter compound then undergoes the normal rearrangements with alkali to give a hydrocellulose terminated by a *meta*saccharinic acid (XLVII). Because of the absence of a carbonyl group, this type of hydrocellulose molecule is stable towards alkali. It is considered that this type of reaction also occurs in the simple cases such as the 4-*O*-substituted monoses but, because of the nature of the stystem, it has not been detected.

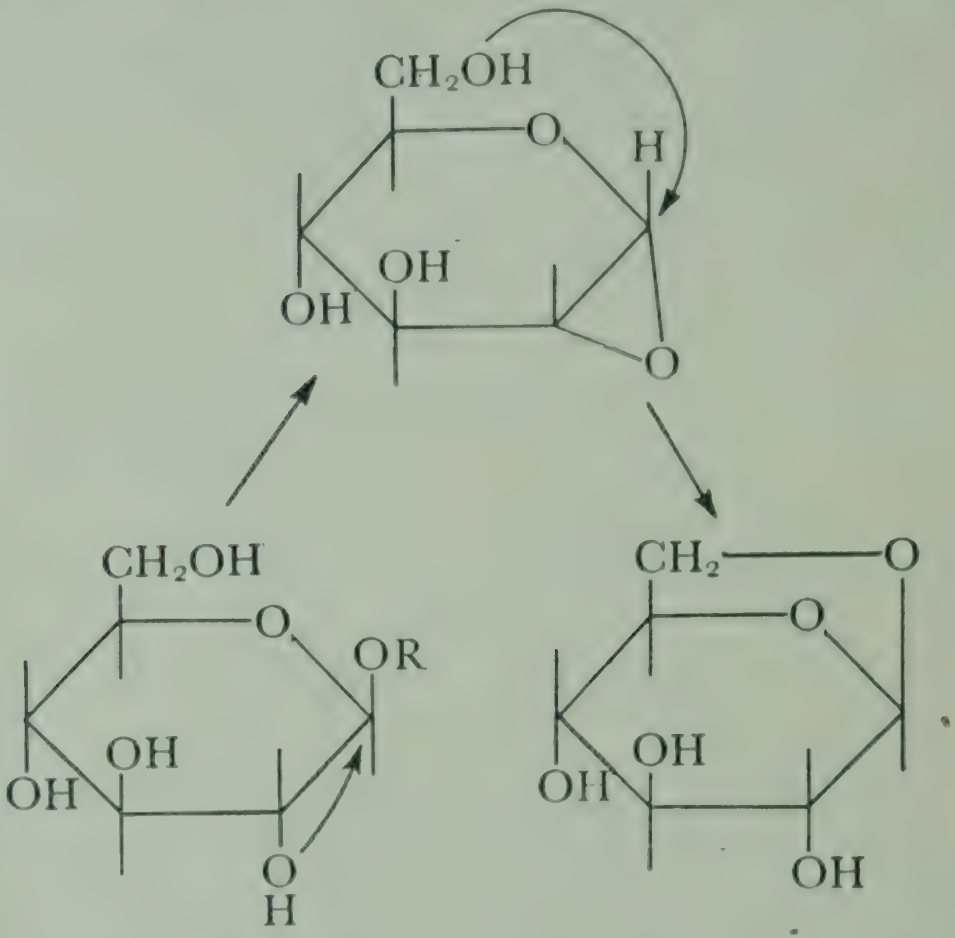


(XLVI)

(XLVII)

Examination of an alkali-stabilized-limit hydrocellulose (i.e. completely crystalline hydrocellulose) appears to indicate that not all of the reducing terminal groups must be converted into a saccharinic acid before the hydrocellulose acquires stability towards alkali. The implications of this are at present unknown.

The reactions just described are those which occur at temperatures of up to about 150°. They have little effect on the degree of polymerization (D.P.) of the material.[27] However, at temperatures of around 170° and above, a further reaction occurs,[28] and this causes chain scission as is shown by a rapid fall in D.P. and a greatly increased weight loss. It has been shown[29] that this second reaction is not due to oxidation by residual traces of oxygen in the system, or to thermal effects alone, but to the action of hydroxyl ions at higher temperatures. Lindberg[30] has studied the behaviour of model compounds under these more severe conditions and, because of the isolation of 1 : 6-anhydrosugars, has postulated that the mechanism of chain scission is similar to the alkaline hydrolysis of phenyl β-glycosides. The hydrolysis of these glycosides[31] occurs by attack of the $C_{(2)}$ hydroxyl ion at the anomeric carbon atom to give a



1 : 2-anhydrosugar which is converted into the corresponding 1 : 6-anhydrosugar by attack of the $C_{(6)}$ hydroxyl group.

Whatever the mechanism of chain scission, it is apparent that the net result is to produce further sites for a stepwise degradation by the alkali in a manner similar to that which occurs at lower temperatures. This reaction is primarily responsible for the weight loss. Thus the weight loss is inversely proportional to the D.P. of the final degraded material, and the acidic degradation products have a similar composition to the products produced at lower temperatures.[31] The occurrence of this scission reaction is of considerable importance in the Kraft process for the manufacture o wood pulp. Also of importance in this process is the recently observed transglycosidation reaction[32] by which hemicelluloses, e.g xylan, may undergo chain scission followed by transference to cellulose molecule.

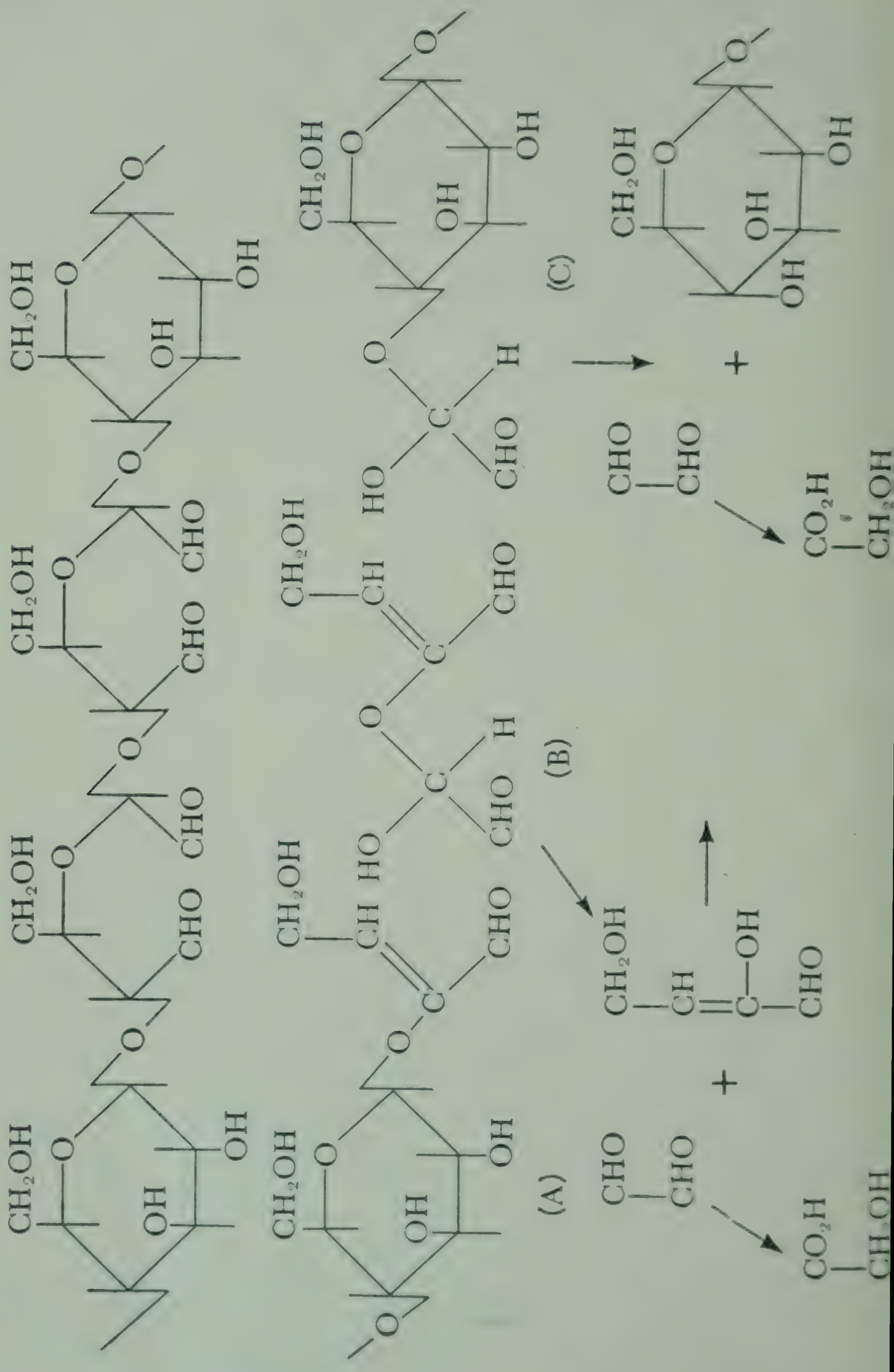
Periodate-oxidized Cellulose

Of the three mechanisms proposed to explain the rapid alkaline degradation of oxidized celluloses, particularly periodate-oxidized cellulose, it has been shown that only one, the β-alkoxycarbonyl elimination mechanism, satisfactorily explains the degradation of the model compounds. In order to establish that this type of reaction is also responsible for the alkaline degradation of periodate-oxidized cellulose, a thorough study has been made of the resulting products.[22a,33] Table 4 lists the postulated initial and final degradation products of periodate-oxidized cellulose according to the three proposed mechanisms. It will be observed that identification of the initial and final products would prove the nature of the operating mechanism. Analysis of the products indicated that the degradation is proportional to the degree of oxidation, and that the degradation proceeds almost completely to acids. These acids were shown to be: (a), volatile acids (*ca.* 20 per cent; mainly formic acid); (b), glycollic acid; (c) α-γ-dihydroxybutyric acid; and (d), acids (*ca.* 20 per cent) of low mobility in paper chromatographic solvents which, because of their reaction with an acidic solution of 2 : 4-dinitrophenylhydrazine, were concluded to be Cannizzaro reaction products.

Table 4: Postulated degradation products of periodate-oxidized cellulose

Mechanism	Postulated degradation products	
	Initial	Final
Evans	Glyoxal D-Erythrose	Glycollic acid $C_{(4)}$ and smaller acids
Pacsu	Glycollic acid D-Erythrose	Glycollic acid $C_{(4)}$ and smaller acids
β-Alkoxycarbonyl elimination	Glyoxal α : γ-Dihydroxybutyric acid	Glycollic acid α : γ-Dihydroxybutyric acid

The isolation of glycollic and α : γ-dihydroxybutyric acids as the two major components, together with the earlier isolation of glyoxal by Head,[21] strongly indicates that a β-alkoxycarbonyl elimination mechanism is the major reaction responsible for the alkaline degradation. Formation of formic acid cannot be satisfactorily explained. The degradation presumably occurs according to the following scheme.


(A)
(B)
(C)

Of the three initial products of degradation (scission reaction) there is evidence that (A) will be stable towards the action of further alkali (cf. the cases of 2 : 3-di-*O*-methyl-D-glucose and the periodate oxidation product of methyl 4 : 6-*O*-benzylidene-α-D-glucoside). The product (B) is an unsaturated hemi-acetal of glyoxal which by further rearrangement will split into glyoxal and an enol, the dicarbonyl form of which will undergo a Cannizzaro reaction to give α : γ-dihydroxybutyric acid. The glyoxal will also undergo further reaction to give glycollic acid. Product (C) is also a hemiacetal of glyoxal which will be hydrolysed to glyoxal and a cellulose molecule terminated at the non-reducing end by a D-glucose unit.

For an oxidized cellulose of low degree of oxidation, products (A), (B), and (C) will be of comparable importance, but for high degrees of oxidation, product (B) becomes more important than the other two. As (B) is the sole source of α : γ-dihydroxybutyric acid it would be expected that the ratio of glycollic acid to α : γ-dihydroxybutyric acid decreases as the extent of the oxidation of the initial product increases. This indeed has been found to be the case.

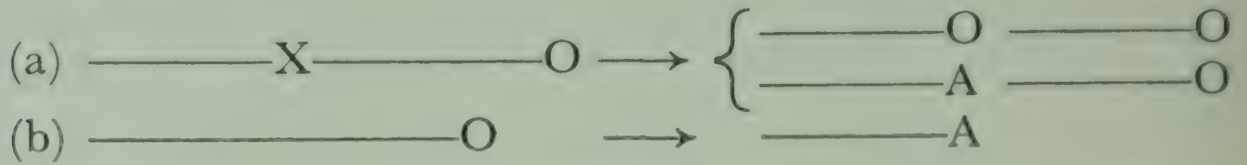
Other Oxidized Celluloses

One of the major acidic products resulting from the alkaline degradation of bromine and permanganate oxidized celluloses has been identified as *iso*saccharinic acid.[34] The latter oxidized cellulose also yielded formic, acetic, lactic, and α : γ-dihydroxybutyric acids. These acids are typical of those produced by the alkaline degradation of cellulose itself. The action of alkali upon permanganate-oxidized cellulose appears to cause chain scission to give acids of unknown nature, and the resulting cleaved cellulose molecule then undergoes alkaline degradation by the normal stepwise mechanism to give lactic, *iso*saccharinic, and other acids.

CONCLUSIONS

A study of the products of alkaline degradation of many oxycellulose model compounds, and also of hydrocellulose, cellulose, and periodate-oxidized cellulose, has established that one reaction, and perhaps the major one, responsible for alkali lability is a β-alkoxy-carbonyl elimination reaction. It must be emphasized that our present knowledge of the reaction does not allow the explanation of volatile acids, particularly formic acid. In order to understand the formation of these acids, further work is required on the action of various alkalis under various conditions upon α- and β-alkoxycarbonyl systems and upon α-β-dicarbonyl compounds.

Our present picture of the action of alkali upon a partially oxidized cellulose molecule is: (a), attack by alkali at the oxidized glucose unit resulting in chain scission by a β-alkoxycarbonyl elimination mechanism; (b), attack by alkali upon the cleaved cellulose molecules of lower D.P. by a stepwise degradation until the molecule becomes stable to alkali by the occurrence of a 'stopping' reaction.



(X represents an oxidized glucose unit in a cellulose molecule; O represents the reducing end; A represents an alkali-stable end unit.)

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5

THE CRYSTAL STRUCTURE OF CELLULOSE

CELLULOSE is distinguished among the fibre-forming high polymers by the variety of its crystalline modifications. For the material itself, i.e. perfectly dry cellulose, there are four recognized crystal structures, Cellulose I, II, III, and IV, which occur or can be obtained under different circumstances; each gives its own characteristic X-ray diffraction pattern. Cellulose I is the modification normally found in the native cellulose of plants, whilst cellulose II is that which occurs in regenerated cellulose (viscose rayon, Cellophane) or can be derived from cellulose I by certain swelling treatments, the best known being mercerization. Cellulose III is formed by treatment of native or regenerated cellulose in liquid ammonia[1] or in dry ethylamine,[2] and cellulose IV is obtained by certain treatments at high temperatures.[3]

Besides these crystalline modifications of cellulose itself, there exist numerous crystalline ' addition ' compounds (water cellulose, the soda-celluloses) and, of course, derivatives such as nitrates and acetates; each presents its own structural problem which has been solved more or less satisfactorily in certain cases.

CELLULOSE I

Of them all, however, cellulose I has been the favourite subject for attention by structure analysts, with cellulose II a close second. The first attempts[4] to elucidate their structures date from the 1920s, but in spite of 30 years' work completely satisfactory answers have not yet been given. The problems which arise in this sort of work are so well exemplified by cellulose I that we shall find it useful to examine this modification in detail.

We start with the generally accepted idea that the cellulose chain molecule can be regarded as a linear repetition of the cellobiose residue. Modern opinion[5] favours the strainless puckered chain form of the pyranose ring, with all the carbon atom bond angles tetrahedral or approximately so, and the same angle of approxi mately 110° between the bonds in the ring and glucosidic oxygen atoms (see Chapter 2). There is, however, a degree of uncertainty in the exact configuration of the cellobiose residue because of the possibility of free rotation about the glucosidic oxygen bonds, so that steric conditions are probably not alone sufficient to determine the form of the cellulose chain. In the most widely favoured structure for cellulose I, that put forward by Meyer and Misch[6] in 1937, the ' symmetrical ' configuration is assumed. In this, Figure 1, the carbon atoms C_1, C_3, C_5, C_1', C_3', and C_5' lie in a plane and all the glucosidic bonds and all the glucosidic oxygen atoms are in a plane perpendicular to this. For fixed atomic distances and bond angles this configuration appears to be the longest possible; according to Meyer and Misch, bond lengths 1·54 Å for C—C and 1·45 Å for C—O lead to a length of just over 10·3 Å for one cellobiose residue. In their model, three successive glucosidic oxygens in the cellulose chain form an isosceles triangle with base angles 13° 47′. Peirce[7] has suggested an alternative configuration for the cellobiose residue in which the planes containing C_1, C_3, and C_5 and C_1', C_3', and C_5' are not parallel, and with bond lengths about 2 per cent less than those of Meyer and Misch he claims to get the same residue length; the glucosidic oxygens form a zigzag of angle 20° 22′ in his model. Part of the discrepancy between these results may be due to the limited accuracy of Meyer and Misch's tabulated co-ordinates since, for example, these lead to a distance of 1·63 Å between C_1 and C_2, although they claim to have used 1·54 Å in their calculations.

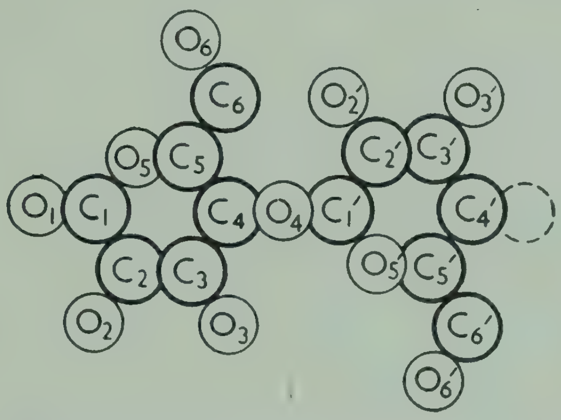

Figure 1. ' Symmetrical ' configuration of the cellobiose residue

The crystal structure of Meyer and Misch,[6] which is a revision of an earlier one due to Meyer and Mark[4] (perhaps the first attempt at a crystal structure for any fibre), is based on a unit cell with dimensions $a = 8{\cdot}35$ Å, $b = 10{\cdot}3$ Å (fibre axis), $c = 7{\cdot}9$ Å, $\beta = 84°$, he angle β being that between the *a*- and *c*-axes which are both perpendicular to the *b*-axis. It should be pointed out that in accordance with accepted crystallographic usage, the *c*-axis should be taken in the opposite direction, so that the angle β should then be given as 96° (180°–84°). Unfortunately, Meyer and Misch's unit cell has been so widely publicized and accepted that there is little hope now of enforcing this convention in this particular case. To attempt to do so would only add to the confusion which already exists in the use of the Miller indices (see below) of the paratropic planes in the crystal (the indices of the (101) and the (10$\bar{1}$) planes would be interchanged) and would worsen the already serious position which has been allowed to develop in connection with the description of the kind of hydrogen bonding which is present in cellulose. This will be referred to again at the end of this paper; meanwhile, we shall continue to use the Meyer and Misch convention. It is well to bear in mind, however, that some authors[8,9] use the other, and that care is necessary in interpreting references to these paratropic planes.

Except for certain suggestions that the Meyer and Misch cell is, in fact, a submultiple of the real cell, to which we shall be referring again later, no serious doubts exist as to its essential validity. There have, however, been numerous reports of slight differences in the X-ray diffraction patterns of various celluloses,[9] and slight variations in the unit cell dimensions may be possible in cellulose from different sources. However, in this paper attention is restricted to the cell given above.

The length of the unit cell in the direction of the fibre axis is 10·3 Å, the length of a cellobiose residue. Two such residues placed in the cell will give a density of approximately 1·59 g/cm^3, which is near enough to the density of solid cellulose fibres (1·55 g/cm^3) to be acceptable. Meyer and Misch arranged the cellulose chains to run through the cell as shown in Figure 2.

We shall examine now some of the details of the Meyer and Misch structure, and see how they fit in with the observed X-ray diffraction results. The arrangement of the chains in the cross-section of the unit cell perpendicular to the *b*-axis is shown in Figure 3. A cellulose chain is located at each corner of the cell, and another is in the centre. (Three of the corner chains really belong to neighbouring cells, so that there are only, in fact, two chains pe cell.) This feature of the structure will lead to certain intensit effects in the reflections on the X-ray diffraction photograph. I order to be able to describe these we must digress for a moment t describe the nomenclature used for referring to the reflections. Th is based on the idea that the interaction of a beam of X-rays with crystal causes the X-rays to be deflected as if they were reflecte (as in a mirror) by certain 'lattice planes' in the crystal. Takin

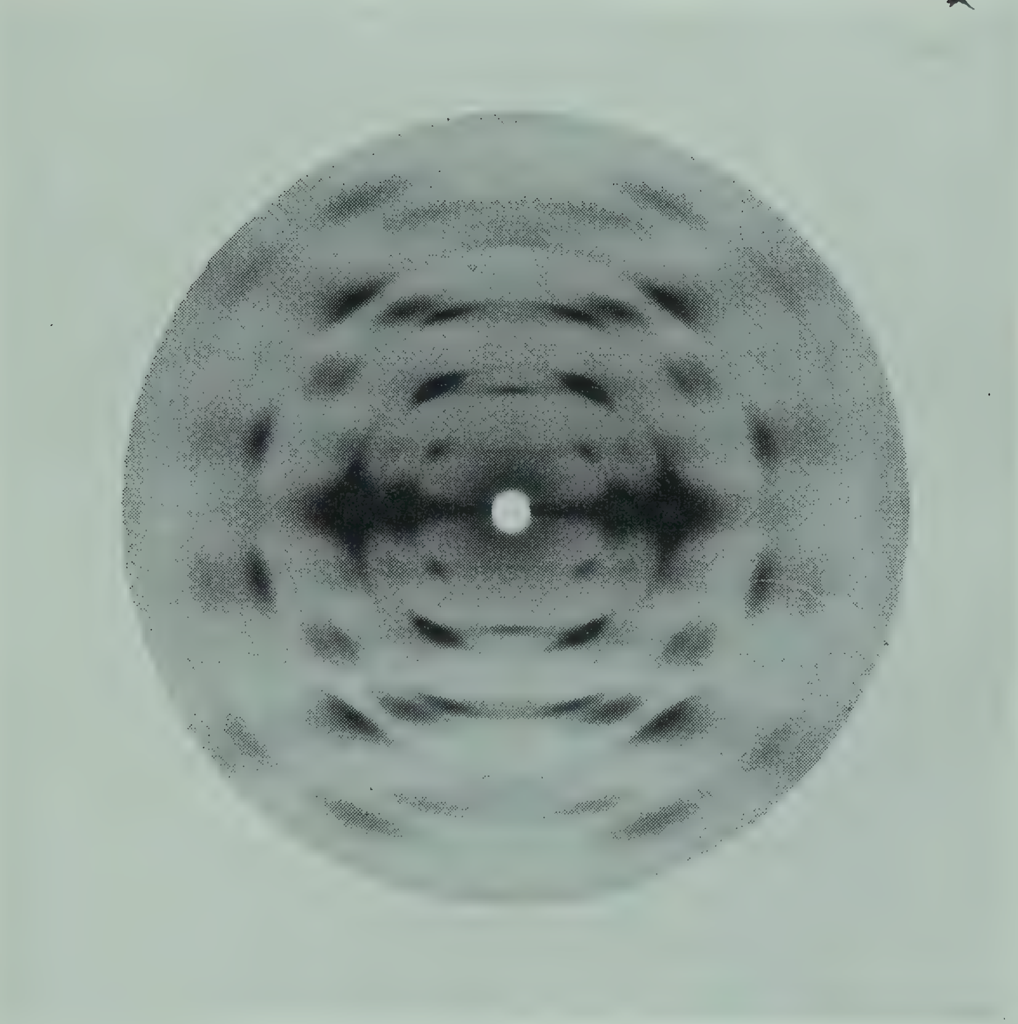

Figure 4. X-ray diffraction photograph of ramie

co-ordinate axes along three concurrent edges of the unit cell we can locate any plane drawn in the crystal by specifying the intercepts which it cuts off along the axes from the origin. The lattice planes form families whose members are parallel and equally spaced; if one member of the family goes through the origin the next one makes intercepts on the axes which are integral submultiples of the lengths of the unit cell edges : a/h, b/k, c/l, where (hkl) are a set of integers called the Miller indices of the planes. The (hkl) planes of a crystal will reflect the X-rays in the way mentioned above only if the X-rays make a definite angle (one of a limited number of possible angles) with the plane. Each spot on an X-ray diffraction photograph such as Figure 4 arises from the reflection of the X-ray beam in a lattice plane family; the spots are therefore referred to by the appropriate Miller indices. In the particular case when one of the Miller indices is zero, the lattice planes are parallel to the corresponding axis of the unit cell; for example the $(h0l)$ planes (Figure 5)

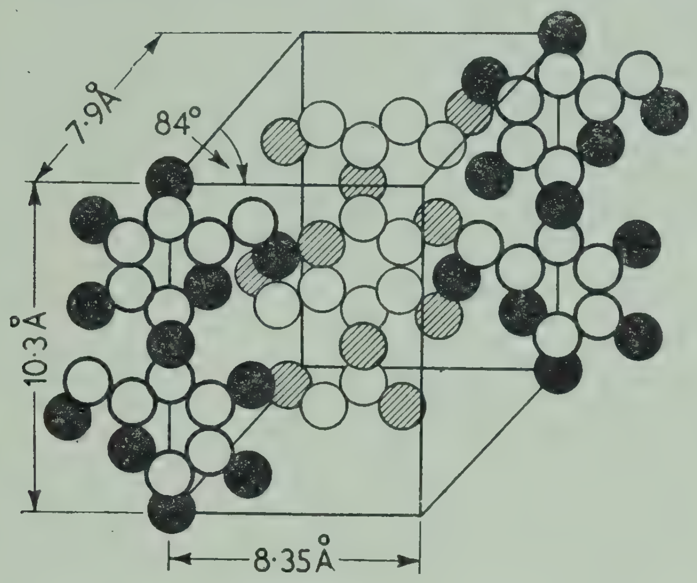

Figure 2. The unit cell of cellulose I according to Meyer and Misch

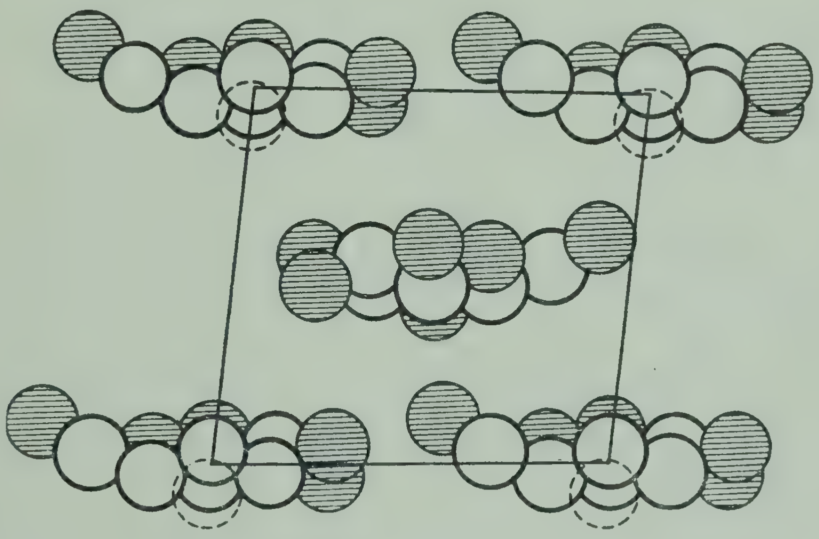

Figure 3. Meyer and Misch cell, basal plane (cellulose I)

are all parallel to the *b*-axis of the cell, whatever the values of *h* and
The X-ray reflections from these planes will all lie on the equat
of the photograph in Figure 4, that is, on the horizontal axis
symmetry of the diagram.

The ' centred ' property of the structure of Meyer and Mis
mentioned above ensures that reflections on the equator only occ
with any intensity if $h + l$ is even. It will be seen from the figu
that there are three very strong reflections on the equator, and
terms of Meyer and Misch's unit cell these are indexed (going o
from the centre) as (101), $(10\bar{1})$, and (002), with $h + l = 2, 0,$ and
respectively. This feature of the structure thus has some justificatio

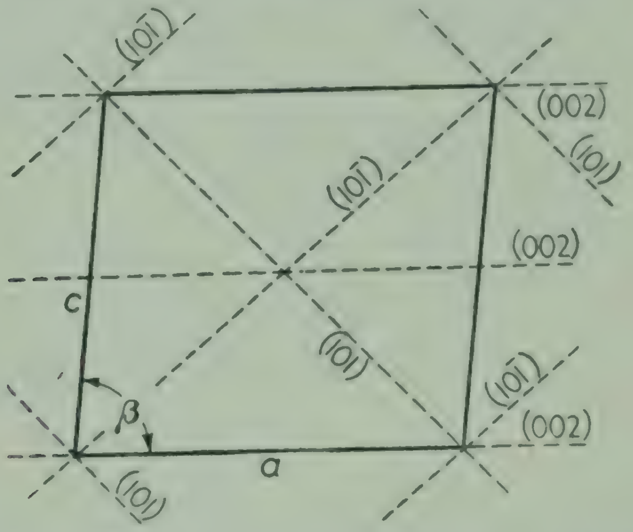

Figure 5. Principal paratropic planes in cellulose I

A second feature which is clearly shown in Figure 3 is that t
cellulose chains are so arranged in the cell that there is a hi
concentration of atoms in planes parallel to the *a–b* face of the ce
with regions of lesser density intervening. Such a distribution
density means that the (002) reflection due to planes parallel to t
a–b face should be very strong, and it will be seen that in Figure
the (002) reflection is, in fact, by far the most intense on t
photograph.

We have noted above that the longitudinal repeat of the crys
pattern is 10·3 Å; this necessitates a chain configuration with t
cellobiose residue having that length, and, as we have seen, Me
and Misch have chosen a configuration in which this condition
approximately satisfied. However, a further restriction on t
possible configurations is imposed by certain intensity relati
among the meridional reflections. An examination of Figure
shows that the reflections are arranged on a set of ' layer lines '—
equator and a series of hyperbolae which cut the meridian
various distances from the centre. The reflections on any layer l
have identical *k*'s in their Miller indices (*hkl*). The meridio

reflections (0*k*0) are located where the layer lines cut the meridian. (Strictly speaking, all the (0*k*0) reflections should be absent if the crystallites in the fibre are all parallel to the fibre axis, if the fibres in the bundle used as specimen are parallel, and if the X-ray beam (of negligible divergence) is perpendicular to the fibres. None of these conditions is strictly observed in practice. The accurate positions of the resulting reflections are just inside the layer lines, not exactly on them.) Now in the photograph, (020) and (040) appear fairly strongly, but (010) and (030) are absent or very weak; this suggests that the (0*k*0) reflections are only strong if *k* is even. If this is so, the crystal structure must have a diagonal screw axis parallel to the *b*-axis of the cell. The existence of such a screw axis means that if the crystal is rotated about it through 180° and then translated along it through a distance *b*/2, it will attain a position identical with that from which it started. In the Meyer and Misch structure it is assumed that the screw axes coincide with the lengths of the cellulose chains; this means that each chain must also have screw symmetry. Hence the ‘ planes ’ of the pyranose rings must be parallel or equally inclined to the screw axis, and this limits the freedom of rotation about the glucosidic bonds. The question thus arises whether it is possible to have more than one configuration satisfying these conditions. Hermans[10] says that it is, but if Meyer and Misch are correct in their claim that the ‘ symmetrical ’ configuration is of the right length, this is not easy to accept. Difficulties arise, however, in the Meyer and Misch structure, for if their tabulated atomic co-ordinates are used the distance $C_6—O_2'$ is only about 2·7 Å (according to Peirce's co-ordinates it is even less than this) which is rather small for van der Waals' interaction. It appears, therefore, that some modification of their configuration is required, if only on these grounds.

Claims have been made[7] that the whole idea of screw symmetry is erroneous, because when intense X-ray diffraction photographs are examined there does appear to be some scattering in the (010) and (030) positions. Legrand,[11] who examined this question very carefully, came to the conclusion that what is seen under these circumstances is not the (0*k*0) reflection but a smearing of nearby off-meridional reflections so that the layer line appears as a streak. This sort of effect could result, for instance, from certain kinds of distortion of the crystal structure. Whether Legrand is right or not, it remains a fact that the odd-order meridional reflections cannot be allowed to have intensities comparable with those of the even-order reflections, so that the departure from screw symmetry is probably not serious.

It will be noted that in Meyer and Misch's structure the chain molecules form two interleaved systems, with those at the corners of the cells in one and those at the centres in the other; Meyer and Misch assumed that the chains in the two families run in opposite directions. If this is so, the screw axes, if present, must coincide with the lengths of the chains. The alternative, that the chains have no screw symmetry, but that the two families are similarly directed, could happen if the screw axes were located midway between the chains in the diagonals of the unit cell base. The consequences of this have not been worked out in detail.

Another feature of the X-ray diffraction pattern must be noted. We have seen that the intensities of the (020) and (040) reflections are too great to be neglected, but careful examination, under the correct conditions for estimating the intensities, shows that the (040) reflection is considerably stronger than (020). The calculated relative intensities of these reflections can be changed by displacing the two families of chains longitudinally with respect to each other. Meyer and Misch claim that agreement with experiment is obtained when O_1 in the centre chain is about 3 Å in the direction of the *b*-axis from O_1 in the corner chain.

Finally, we can say something about the kind of hydrogen bonding which might be expected in the Meyer and Misch structure. The chains are, as we have said, arranged with the pyranose rings parallel to the *a–b* plane; under these conditions the secondary hydroxyls of one chain approach to 2·6 Å and 3·1 Å from the primary hydroxyl of the next. This suggests the possibility of hydrogen-bonding between these hydroxyls with, perhaps, a slight adjustment of the position of the primary hydroxyl to make the two distances more nearly equal. Thus we may suppose that the chains are linked together into sheets in the *a–b* planes by these hydrogen bonds. It is worth noting here that various workers in the infrared[12,13] absorption field have claimed that their results indicate that all the hydroxyl groups in cellulose are hydrogen bonded, since the observed absorption frequencies are not those to be expected from the vibrations of completely free hydroxyl groups. Hydrogen bonding of the kind described above is not at variance with this observation.

On the whole, the suggested structure of Meyer and Misch has shown itself capable of explaining many of the more obvious features of the X-ray diffraction pattern. The authors claim tha the quantitative agreement between the observed and calculated intensities is as good as can be expected in view of the somewha diffuse character of the reflections and the fact that a good deal o superposition of reflections occurs. Nevertheless, there remain enough doubt as to the validity of their structure to tempt other workers to try to improve on it. We shall, therefore, examine briefly some of the criticisms, favourable or otherwise, of the Meyer and Misch structure. Firstly, apart from the success claimed by Meyer and Misch themselves, some subsequent investigations[14,15] have seemed to be in favour of their ideas. For example, Oberlin and Mering[15] find reasonable agreement between the radial distribution of interatomic vectors in the Meyer and Misch model and that calculated from the observed intensities in the X-ray diffraction photograph. On the other hand, Norman,[16] from the results of somewhat similar work, concludes that some modification of their structure is necessary. The more definite adverse criticisms are detailed below.

The Chain Configuration

We have noted some difficulties which are still unresolved in this connection. The most serious objections otherwise are due to Peirce,[7] who rejected the idea of the strainless puckered pyranose ring in favour of a nearly flat strained arrangement of the carbons but with the ring oxygen out of the plane. Peirce thought that this configuration was more likely than the other because it allowed the possibility of intra-chain hydrogen bonds without the pronounced zigzagging which is so apparent in the Stuart[10] chain. Peirce's suggestion leaves some of the hydroxyls unbonded and Mann and Marrinan[13] therefore reject his ideas as being at variance with the infra-red absorption results.

The Screw-axis

Peirce's main argument was that there was only ' pseudo-screw-symmetry ' and he used the idea of chelated hydrogen bonds in one set of chains in order to destroy the screw symmetry. Since Legrand's interpretation of the X-ray diffraction results is now generally accepted, this notion of Peirce's has fallen into disrepute. However, the question of chain configuration must still be regarded as capable of further clarification, particularly on account of the possibility of steric interference in the Meyer and Misch model.

The Chain Directions

The question of the alternating chain directions is still unanswered; Meyer[17] himself has allowed the possibility that a random ' heads and tails ' arrangement of the chains might occur without disturbing the ' crystallinity ' too seriously. Recently, Dulmage[18] has raised the question again in connection with the structure of cellulose triacetate. Meyer and Misch[6] originally used an erroneous argument to justify their postulate of alternation; later, they claimed that no justification is really necessary if the answer is correct, a sound argument *if* the answer is correct!

The Unit Cell

Various authors[19,20] have criticized the Meyer and Misch unit cell on the grounds that they have observed reflections in the X-ray diffraction pattern which cannot be indexed in terms of this cell. Usually, the ' abnormal ' reflections would fit in if one or both of the *a*- and *c*-axes of the cell were to be doubled, leaving the angle β unaltered. Sometimes these claims have been rejected[21,22] as being due to faulty experimentation, but recently the question has been raised again by Honio and Watanabe,[23] who have investigated cellulose fibrillar sheets from *Valonia* by electron diffraction at low temperature. They claim to have observed many more ' reflections ' than appear in the conventional X-ray diffraction pattern, and a number of these can only be indexed if both *a*- and *c*-axes of Meyer and Misch's cell are doubled. This suggests that there may be an alternation in the chain configuration along the *a* and *c* axes as well as diagonally. The scattering of the alternate chains might then be similar enough to give the correct intensities to the Meyer and Misch approximation, but small differences might exist which can only be detected using more refined techniques.

CELLULOSE II

It should be pointed out here that the structures of cellulose I and II must be consistent in the sense that it must be possible to pass from one to the other (or at any rate from I to II) without complete disruption of the crystallites. The question of chain directions, for instance, must be considered in the light of the possibility of this transition, which takes place on mercerizing native cellulose fibres. Before going on to discuss the last unresolved difficulty in the Meyer and Misch structure, it is necessary to say something about the structure of cellulose II, although it is not proposed here to go into all the details, but only to describe briefly the salient features of what is accepted, at the moment, as the most satisfactory structure so far put forward.

The principal features of this, due to Andress,[24] are illustrated in Figure 6. The unit cell, $a = 8{\cdot}1$ Å, $b = 10{\cdot}3$ Å (fibre axis), $c = 9{\cdot}1$ Å, $\beta = 62°$, is of almost the same volume as that of cellulose I, and since it also contains two cellobiose residues the density must be approximately the same. The pyranose rings of the cellulose chains are rotated so that they are more nearly parallel to the $(10\bar{1})$ planes than to the (002) planes; the chains at the corners and centres of the cells form two families with reverse chain directions, and the centre chains have been displaced longitudinally from their positions in cellulose I to allow hydrogen bonding to take place in the diagonal direction. The hydrogen bonds thus link the chains together into sheets parallel to the $(10\bar{1})$ planes. (It is sometimes said that in cellulose II the hydrogen bonds are in the $(10\bar{1})$ planes, but this is not strictly true and, for reasons which will appear later, statements of this kind should be carefully made if they are not to lead to misunderstandings.)

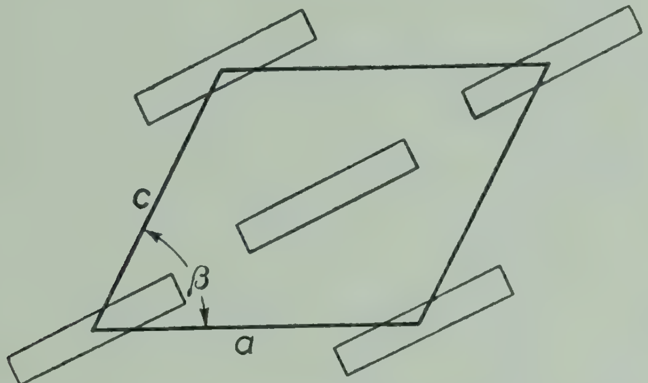

Figure 6. Andress' cellulose II unit cell, basal plane

It has usually been assumed that the chain configuration is the same in cellulose I and II. Recently, however, Petitpas and Mering[25] have examined the radial distribution of interatomic vectors in cellulose II and find that the Meyer and Misch configuration, which Oberlin and Mering[15] had previously accepted as reasonable for cellulose I, gives unsatisfactory agreement with experiment in cellulose II. They therefore suggest an alternative configuration, in which the pyranose rings have been rotated about the glucoside bonds. The effect of this is to make the chain assume a more zigzag character, and it is not quite clear how much reduction in the repeat length this will entail. As we have noted above, some slight reduction might be allowed, or even necessary; clearly, however, the time is ripe for a re-examination of this question.

CRYSTALLITE HABIT IN CELLULOSE

A problem which is closely tied up with the nature of the crystal structure and with questions of fine structure which will be dealt with in the next chapter, is that of the habit of growth and the possibility of preferred cleavage of the crystallites. For a long time it has been known[26] that in regenerated fibres and films, orientation effects occur which suggest that the crystallites are laminar o ribbon-like in shape, with the largest surface parallel to the (101 planes of the crystal. For instance, in sheet cellulose of the Cello phane type the normals to the (101) planes are found to be roughl perpendicular to the film surface. Now if we assume that during th formation of the regenerated cellulose some growth of the crystallite has occurred (some authors claim that during the regeneratio process the cellulose is completely dispersed, but it is not necessar here to insist on this) the observations suggest that the rate of growt is slowest perpendicular to the (101) planes. This is, in fact, entirel reasonable if the crystallization is taking place from a solution i which the cellulose chains are solvated, for the mutual attraction o the hydroxyls in different chains would then be small, whereas th van der Waals' attractions would be relatively unaffected. Thu growth is slow in the direction perpendicular to the (101) planes the direction in which chain accretion by hydrogen bonding take place. A crystal face parallel to (101) would be very rich in hydroxy groups and would, in fact, be hydrophilic. Under conditions wher hydrogen bonds are broken, the (101) planes are thus planes o weakness, and might be looked upon as cleavage planes. Upo dehydration, however, the chains are bound together tightly in th direction normal to these planes.

There does not seem to be any doubt about the correctness of thes ideas, and they have been more or less universally accepted (i implicitly) for a long time. Recently, however, somewhat simila considerations have had to be applied to cellulose I crystallites. A far as crystal growth is concerned, work done on the cell walls o the algae[27] shows that generally the (101) planes of the crystallite tend to lie flat in the wall, so that there is reason to suppose that th crystallites are thin in the direction normal to the (101) planes Further, studies of the acid hydrolysis of native fibres have show that the cellulose can be broken down into tablet-shaped particle which tend to sediment on a flat surface with the same kind o orientation[28] (Figure 7). This breakdown effect may, of course, b an example of preferred cleavage rather than of habit of growth o the crystallites, but from the present point of view this is immateria Here, as in cellulose I, the (101) planes are distinguished as bein particularly susceptible to agents which break hydrogen bonds, an the bonding perpendicular to these planes is weak under conditio of strong hydration. Now in cellulose I, according to Meyer an Misch, the hydrogen bonds tend to be parallel to the *a*–*b* planes, an in the dehydrated crystal the bonding in these planes is strong. does not follow, however, that the chains are tightly bound togeth

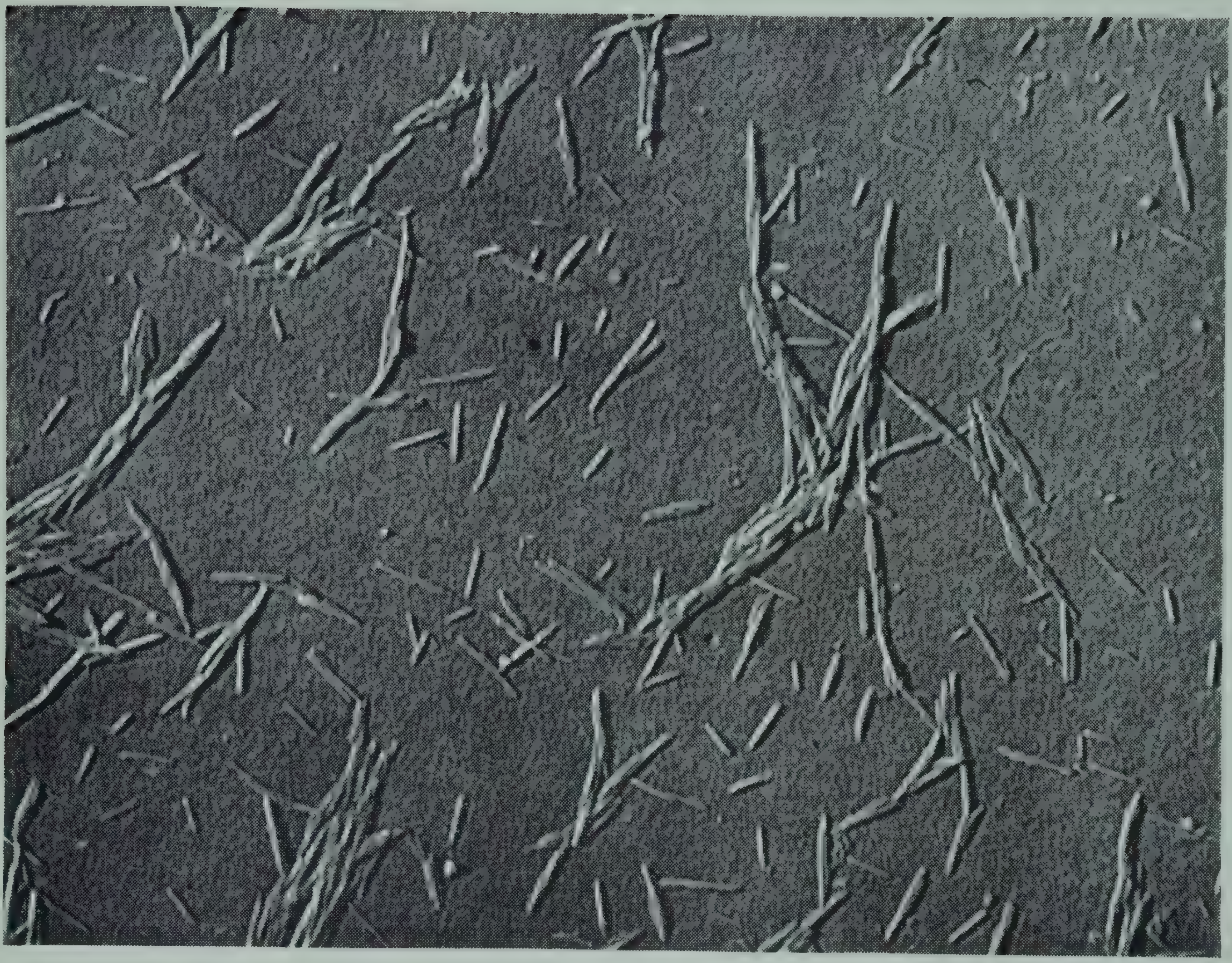

Figure 7. Cellulose particles obtained by acid degradation of native cotton fibres. Electron micrograph (× 58,000). The particles are tabular and have the crystal structure of cellulose I

in these planes in the hydrated material; rather, the contrary will hold. As in cellulose II, we should expect the planes of weakness in hydrogen-bond-breaking media to be those rich in hydroxyl groups; according to Meyer and Misch these are primarily the (101) planes and secondarily the $(10\bar{1})$ planes (cf. Figure 5). The (101) plane will thus, in cellulose I, have analogous properties, in respect of growth and cleavage, to the (101) plane in cellulose II. There does not seem to be any reason, therefore, to postulate the type of bonding favoured by Frey-Wyssling,[29] who wishes to stabilize the (101) planes by having hydrogen bonds between the corner and central chains of the cell. Frey-Wyssling accepts the general picture of the cellulose I structure put forward by Meyer and Misch, but bases his speculations about the hydrogen bonding on an analogy with the structure of silk fibroin[30] rather than using, as we have done here, the parallel of cellulose II. In silk fibroin, as in other fibrous proteins of the β-type, hydrogen bonding between the polypeptide chains leads to the formation of molecular sheets, and in thin films these, and consequently the hydrogen-bond directions, tend to be parallel to the surface of the film. The mechanism of crystallite growth, therefore, is undoubtedly one of chain accretion via the hydrogen bonds, even in the hydrated material. In the proteins and in cellulose II, therefore, the indications, each quite unambiguous, point to diametrically opposite conclusions. Faced with this dilemma, Frey-Wyssling chose one alternative; there is some justification for the assertion that his choice was not the more logical one. Unfortunately, Frey-Wyssling's arguments are tending to be accepted uncritically by other authors, and we can even read now that, as a reason for this suggested bonding in the (101) plane, ‘ the swelling of the lattice with polar hydrogen-bond-breaking reagents gives an expansion in the (101) dimension of the lattice ’ and that ‘ the (101) plane is probably the main hydrogen-bonding plane also in cellulose II ’.[31] The first statement is incorrect, for how can breakage of bonds *in* the (101) planes lead to the observed increase in the chain separation in the direction *normal* to the (101) planes? The second statement is in direct contradiction to the usually accepted cellulose II structure.

A further complication in this already unnecessarily complicated story has recently arisen through an observation by Kreger[32] that, in some algal cell walls, orientation of the $(10\bar{1})$ planes, and, in others, of the (002) planes is present. It is possible, therefore, that Frey-Wyssling's idea may be helpful in explaining these abnormal orientation effects, but if so it would appear to be another case of a correct conclusion being reached by a fallacious argument.

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6

THE FINE STRUCTURE OF CELLULOSE

INTRODUCTION

Because of the relative abundance of cellulose in Nature and its importance as a material in textile and other applications it has been the subject of many attempts to elucidate the finer details of its structure. Many of these investigations were instrumental in advancing the experimental techniques and also our ideas about the structure of long-chain molecules which have proved to be invaluable in the study of other high polymers.

In the progress of these investigations it was realized that the physical and chemical properties of cellulose depend, to a great extent, on the nature of the monomer unit, the way in which the monomers are joined together, and the way in which the resulting molecules packed in the solid state. Thus the study of cellulose has involved the work of many chemists, physicists, and physical chemists in investigating these aspects of structure.

The result of all this work is that we now know that a cellulose molecule consists of anhydroglucose units joined together in the 1-4-β configuration to form long molecular chains and that a certain fraction of these chains, or more precisely certain segments, pack together to form crystalline regions whilst the remaining segments constitute what are called the amorphous or disordered regions. In these amorphous regions the polymer chains will tend to be folded, and perhaps entangled with each other, and consequently they will have rather different properties than the crystalline regions. It is in these disordered regions that most of the chemical reactions with cellulose take place. This dual structure of crystalline and amorphous regions intermixed, coupled with some orientation of these regions, is a characteristic feature of fibres and is known as fibre structure.

The cellulose molecule is perhaps outstanding among high polymers in that it can form a variety of materials with different physical and chemical properties. The reason one cellulosic material differs from another may reside in a different type of packing in the crystalline or the amorphous regions, in a difference in the propor tion of crystalline and amorphous regions, in a difference in thei orientation, or in a difference in a variety of other structura features. Unfortunately, however, the relationship between the bul properties of a material and its intrinsic structure is not by an means fully understood despite the great deal of effort that has gon into it. This is no doubt partly due to our lack of knowledge abou the disordered regions which in many cases constitute the majo part of the material. Other aspects of the structure are, however better understood and can be defined more precisely, and in thi chapter the details and some of the techniques which hav enhanced our knowledge of the fine structure of cellulose will b discussed.

THE CRYSTALLINITY AND ACCESSIBILITY OF CELLULOSE

It has been known since the work in 1913 of Nishikawa and Ono that samples of cellulose contain a certain amount of well-ordered o crystalline material. This was shown to be so when it was found tha the X-ray diagram of cellulose contained several well-defined spot indicating an arrangement of the molecules at least approximatin to that found in crystals of low molecular weight compounds. Th crystallinity in cellulose, however, does not arise from a singl crystal but from a polycrystalline aggregate consisting of smal crystal regions separated by amorphous regions. Many independen investigations have been directed towards the determination of th percentage crystallinity of cellulose because it was assumed fo many years that this was the most important single characteristi affecting the bulk properties of a sample. Even today a great dea of work is still carried out on this subject.

The methods that are used may be divided up into two types Firstly, there are the physical methods, all of which are based on th fact that crystalline cellulose is packed differently from non crystalline cellulose and hence will have different physical propertie Secondly, there are the chemical methods which may be sub divided into two classes. In the first class may be placed method involving kinetic studies of some chemical reaction which proceed initially at a rapid rate, followed by a slower one. The assumptio made here is that the initial rapid rate involves reaction in th amorphous regions and the slower rate involves attack on th crystallites themselves, and that a suitable extrapolation of the slov rate will give a measure of the amount of non-crystalline materia initially present in the sample. A typical method relying upon th assumption is acid hydrolysis.[2,3] In the second class of chemical methods are reactions which rely upon reagents which react with a limited percentage of the total number of hydroxyl groups present. It is assumed here that the reagent is unable to penetrate the ordered regions and the extent of the reaction is a measure of the accessible cellulose present.

The results obtained using these methods show little agreement on an absolute scale, and this has led to much discussion on their relative merits. This indeed is not surprising because each method relies on its own, somewhat arbitrary, definition of what is 'crystalline' and 'non-crystalline'. However, all of the methods agree in placing the cellulose samples examined in approximately the same order of crystallinity.

If it is accepted that the amorphous regions of cellulose consist of varying degrees of order, then it follows that the results obtained with chemical methods will vary with the size of the penetrant molecules used, and the chemical nature of the medium in which the reaction takes place. For this reason it has been recognized that for chemical methods it is better to use the terms 'accessible' and 'inaccessible'. Furthermore, it is believed that the crystalline surfaces as well as the disordered regions are accessible to certain chemical reagents and hence the total accessibility may vary with the size or shape of the crystallites even at constant crystallinity. The relationship between the crystallinity and the accessibility of a cellulose may be expressed[4] by

$$A = \sigma\alpha + (100-\alpha) \qquad \ldots (1)$$

where A is the percentage accessibility, α is the percentage crystallinity, and σ is the fraction of the crystallites accessible to the reagent. Thus, to determine a crystallinity from an accessibility, some knowledge of the size, shape, and arrangement of the molecules in the crystalline domains is required. Our knowledge of cellulose structure at the present time does not allow this to be done with any degree of accuracy. If, however, both the crystallinity and the accessibility of a cellulose are determined separately then equation (1) may be used to obtain an estimate of the fraction of the crystalline regions that is accessible. It is thought that this fraction is most probably associated with the surfaces of the individual crystallites, and if certain assumptions are made about the shape of these regions, a rough estimate can be obtained of the crystallite size. Although this method may be useful for showing up differences in the size of the crystallites in different samples, a great deal of importance should not be attached to the absolute values obtained until our knowledge of the shape and arrangement of the molecules in the crystalli regions is more complete.

Physical Methods for Crystallinity and Accessibility

The first quantitative methods for measuring the percenta crystallinity were proposed by Hermans,[5] and Kast and Flaschner Both methods defined the crystalline regions as those which gi rise to specific reflections producing maxima in the X-ray diagram The percentage of crystalline material was obtained by compariso of the integrated intensity of the maxima and the intensity of th more diffuse scattering which arises from the polymer molecules i the disordered regions. Figure 1 illustrates this procedure wit viscose.

The main criticism often levelled against the X-ray method that since only crystallites larger than a certain size will contribut to the maxima the method can only be regarded as giving a approximate answer. In addition it is apparent that large experi

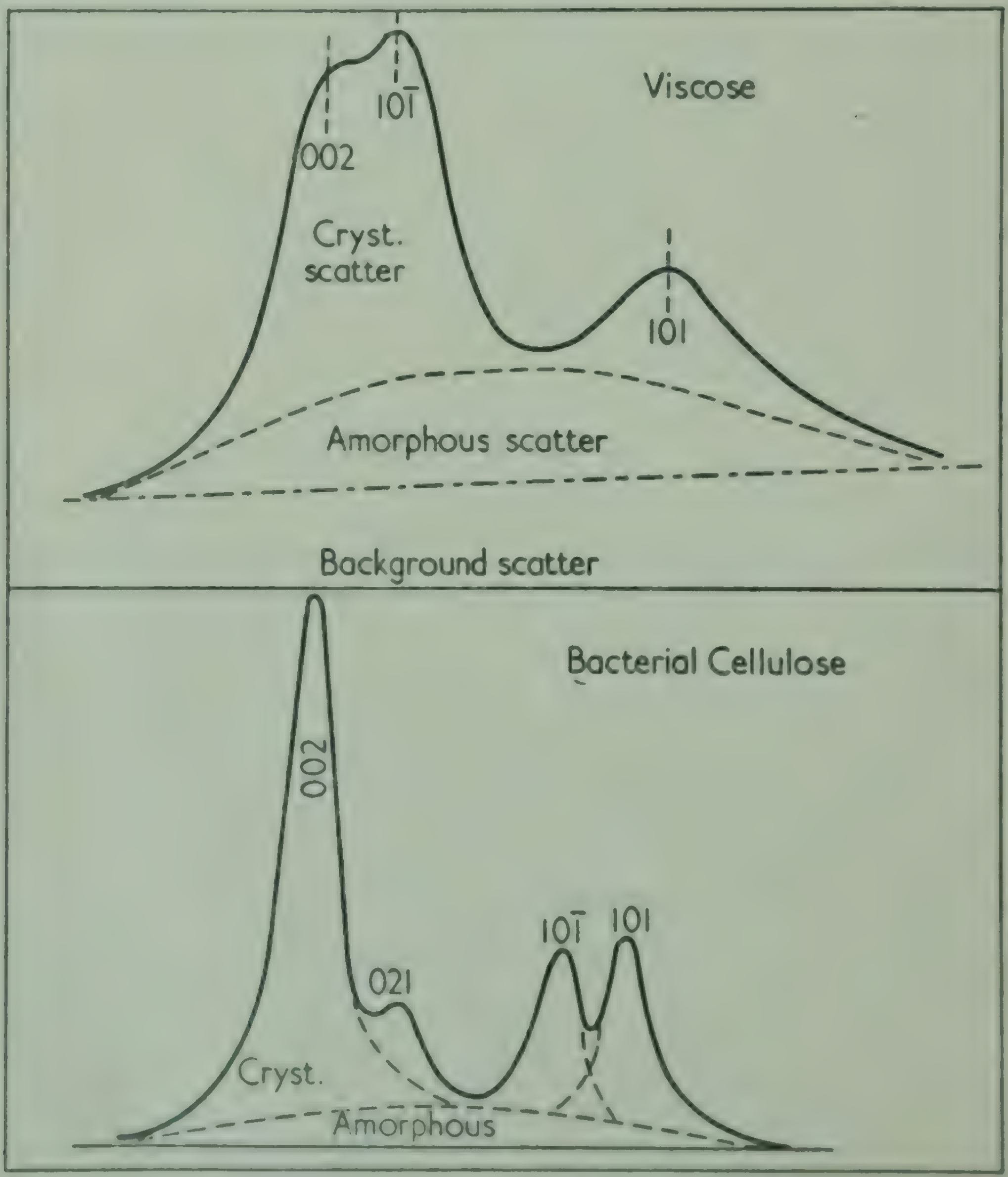


Figure 1. Determination of proportions of crystalline and amorphous material by the X-r method

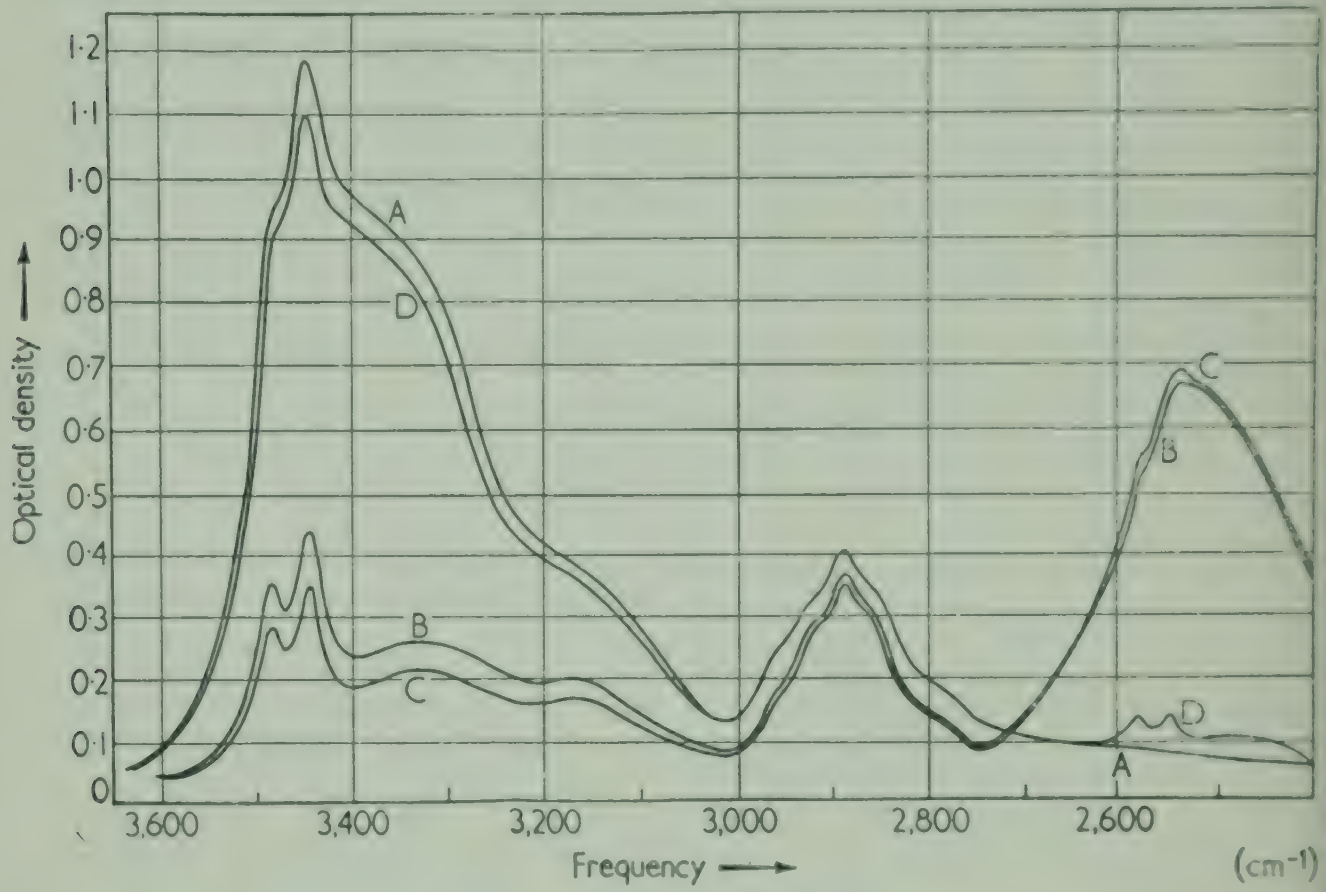
mental errors can arise in this method because of the difficulty of apportioning the composite diffraction curve correctly between the crystalline scattering, the amorphous scattering and the background scattering. The method is, however, quite reliable when the amorphous and crystalline reflections show definite bands, for then it is a fairly straightforward matter to resolve the peaks due to amorphous and crystalline material.

If the specimen being examined is oriented then the determination of the crystalline–amorphous ratio by the X-ray method is a more difficult matter experimentally. The reason for this is that the diffraction pattern of an oriented specimen exhibits more or less discrete reflections and thus an estimate of its crystallinity involves the measurement of integrated intensities along the arcs produced by the crystalline regions and along the amorphous rings. An attempt is sometimes made to randomize the specimen before X-raying by cutting it into short lengths and rotating the resulting samples. This method of averaging is not entirely satisfactory however as it does tend to increase the relative background scattering and thus add to the difficulties of measuring. For this reason it is often assumed that the proportion of crystalline material in an oriented sample is approximately the same as in the unoriented one if the density is the same. Even so the X-ray method is probably the most widely used today and indeed is capable of giving valuable results so long as its limitations are borne in mind. Some of the results[7] obtained by this method for various types of cellulose are shown in Table 1.

Table 1: The crystallinity of various types of cellulose from X-ray diffraction[7]

Material	Percentage crystallinity
Cotton	70·0
Ramie	70·0
Valonia ventricosa	68·0
Bacterial cellulose	40·0
Fiber G	53·0
Fortisan	49·0
Viscose rayons	38·0–40·0
Mercerized ramie	47·0
Mercerized bacterial cellulose	31·0

In recent years, infra-red spectroscopy has been used[8] in conjunction with the deuterium isotopic exchange reaction to measure the crystallinity as well as the accessibility of a number of different celluloses. The advantage of the spectroscopic method over othe methods[4] of following the exchange reaction lies in the fact that th shape of the absorption band due to the hydroxyl groups in th crystalline regions is different from that of the hydroxyl groups in th amorphous regions. Mann and Marrinan[9] demonstrated this b comparing the spectrum of viscose film with the spectra of crystallin cellotetraose and cellopentaose. Thus, whereas previous workers[4,1 using the deuteration technique followed the reaction by rat measurements, these authors showed that the infra-red spectru



A: Dry viscose, undeuterated;
B: Deuterated in deuterium oxide vapour for 4 hr;
C: Deuterated in deuterium oxide liquid for 4 hr;
D: Rehydrogenated in water for 4 hr

Figure 2. Viscose film; exchange with deuterium oxide

could be used to determine when all the hydroxyl groups in th amorphous region had been deuterated, and hence an accurat measurement of crystallinity could be made.

The infra-red spectrum of viscose on exchange with heavy wate and subsequent re-hydrogenation with water is shown in Figure 2 Before deuteration the infra-red spectrum is shown by the soli curve A and the absorption band of the hydroxyl groups is th broad region of absorption stretching from 3,600 cm^{-1} to 3,000 cm This band is produced by hydroxyl groups in the crystalline and th amorphous regions and, as seen, the main effect of deuteration is t remove the broad absorption band of the amorphous region leaving a number of much sharper absorption bands which are characteristic of the crystalline regions as shown by comparison with crystalline cellopentaose. At the same time a very broad almost structureless OD absorption band appears (centred around 2,500 cm^{-1}) contributed by the disordered regions in which deuterium exchange readily takes place.

With liquid deuterium oxide the exchange reaction is almost complete after 4 hr. With deuterium-oxide vapour, however, the reaction does not proceed quite so far and is almost complete after 1 hr. When a viscose film, which has been in liquid deuterium oxide for some hours, is re-hydrogenated in water for at least the same length of time, it is found that the OD band does not disappear completely. These resistant OD groups, as shown by their spectrum, Figure 2, are in fact in the crystalline regions. It has been shown[8] that the presence of these OD groups in the crystalline regions must be a consequence of some molecular rearrangement during the treatment, and is not due to the direct penetration of the heavy water into these regions. This illustrates one of the advantages of following the exchange reaction by infra-red spectroscopy.

From experiments such as these it is possible to determine[8] both the accessibility and the crystallinity of a cellulose. The accessibility to liquid deuterium oxide may be found from the relative intensities of the OD and OH absorption bands shown in curve C, Figure 2.

In order to determine the crystallinity, Mann and Marrinan[8] defined the crystalline material in a cellulose sample as that which has a three-dimensional order sufficient to give a regular repeating system of hydrogen bonds. Thus by measuring the percentage of OH groups which are hydrogen-bonded in this way an estimate can be obtained of the crystallinity. It is essential, however, when carrying out this measurement, to use a vapour deuteration technique in order to be able to stop the reaction when all the OH groups in the amorphous regions have been deuterated—this being judged from the overall shape of the band.

The infra-red measurements are sufficient to put the results from any number of samples of cellulose which contain the same crystal structure on a relative scale of crystallinity. In order to put these on an absolute scale the method needs to be calibrated; Mann and Marrinan did this[8] by extracting with water the deuterium that had exchanged with the cellulose and determining the amount by measuring the refractive index of the mixture. Some of the results that have been obtained by these authors for the crystallinity and the accessibility of a number of celluloses are shown in Table 2.

The accuracy of the figures determined by the infra-red method is thought to be ± 1 per cent except in the case of micelles prepared by acid hydrolysis where an accuracy of ± 5 per cent is quoted because these materials scattered the infra-red radiation rather badly.

It is apparent from Table 2 that this method generally gives significantly more amorphous material than the X-ray method as shown by Hermans' measurements[7] of crystallinity. However, the crystallinities defined as above are not of course the same as those used in the X-ray or density methods, since some at least of the OH groups of chains which lie on the surface of crystallites will not absorb in a characteristic crystalline manner. The crystallinities determined by the infra-red method should therefore be lower than crystallinities determined by a method such as X-ray diffraction. This generalization may not hold, however, when the crystallite size is small, since the infra-red method should detect smaller crystallites than the X-ray method. Apart from Hermans' value for bacterial cellulose, which it is now generally agreed has a higher crystallinity than shown in Table 2, the relative crystallinities given by the two methods show good agreement. The Fortisan and cotton micelles, whose crystallinities are shown in Table 2, were prepared by acid hydrolysis and were thought to be completely crystalline,[11] and to be closely related to the crystallites present in the original cellulose. However, as is shown in this table they have 47 ± 5 per cent and 31 ± 5 per cent, respectively, of their OH groups accessible and hydrogen-bonded in an amorphous manner. These figures agree remarkably well with the corresponding accessibilities o

Table 2: The crystallinity and accessibility of various types of cellulose[7,8]

Material	Infra-red method[8]		X-ray method[7]
	Accessibility (per cent)	Crystallinity (per cent)	Crystallinity (per cent)
Saponified acetate	—	25	38
Viscose	78	26	38–40
Precipitated cellulose	—	32	45–53
Viscose treated with 18 per cent sodium hydroxide	70	33·5	52–54
Fortisan micelles	—	53	—
Mercerized bacterial cellulose ...	—	33	31
Bacterial cellulose	36	70	40
Cotton micelles	—	69	—

48 and 32 per cent obtained by Howsmon[12] from water sorption measurements. In both cases the micelle size must be very small if, as is thought, the OH groups which are in an amorphous environment lie in the surface of the micelles, and this will be discussed more fully in a later section.

Although the infra-red method is capable of a high degree of accuracy in determining the amount of the ordered material present in a cellulose specimen it has a limitation in that it is only suitable for thin samples. Thus most of the work up to the present has been done with films of material rather than fibres. However, fibres may be examined if they are aligned side by side,[13] or if some means of compressing a bundle to reduce the total thickness is used.[14]

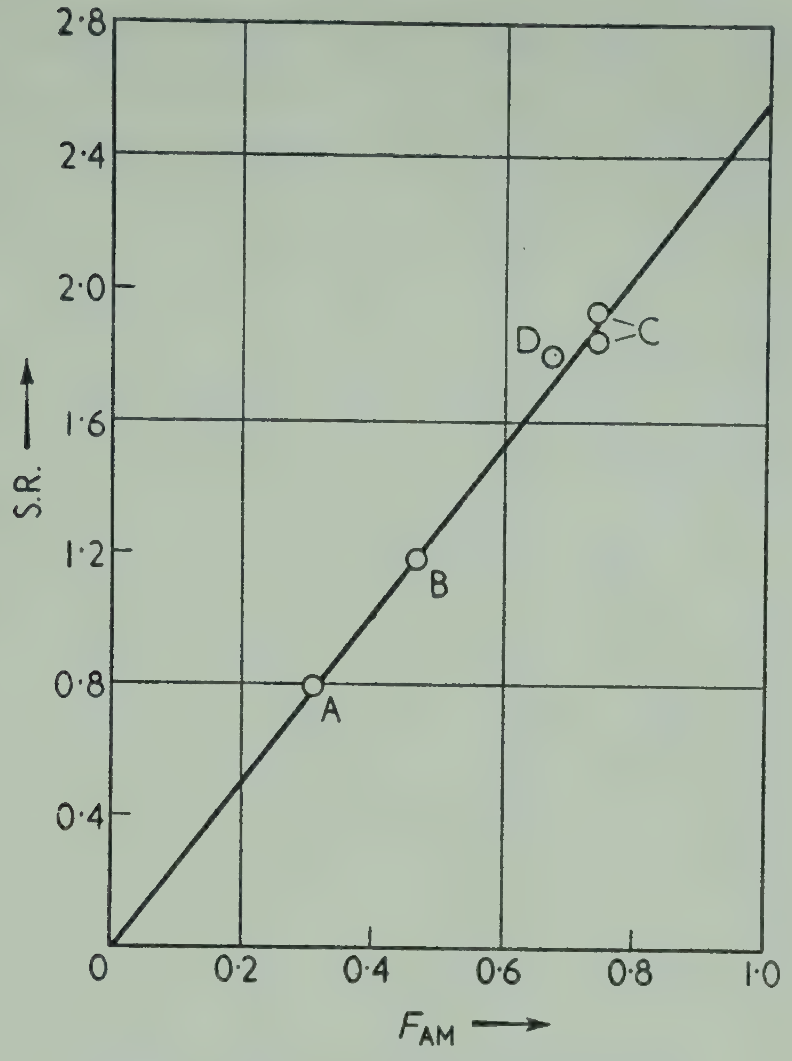
Hermans has proposed[15] the measurement of the density of a sample of cellulose as a means of estimating its crystallinity. Although the density of the crystalline regions can be determined with reasonable accuracy from the unit cell dimensions, there is no means of estimating the density of the non-crystalline regions directly. A second difficulty is in the accurate determination of the volume of the sample and the only feasible method of doing this involves the use of immersion media. Both swelling and non-swelling liquids have been used[16] but even in the latter case the degree of penetration is still determined by the nature of the liquid and the texture of the fibre, and so the method is not absolute. Because of this difficulty it has been suggested that an inert gas like helium should be used and, although Davidson[16] has shown that the estimated density of cellulose is higher using helium than it is using toluene, a precise interpretation of the volume measured is still not possible. If, however, the densities determined by this method are accepted, one must still assume that the amorphous fraction has a constant mean density from sample to sample. If this is done then the weight fractions of crystalline material C_1 and C_2 in two samples can be expressed in terms of their densities ρ_1 and ρ_2 and the density of the crystalline material ρ_0 by the following expression:

$$\frac{1 - C_1}{1 - C_2} = \frac{\rho_2(\rho_0 - \rho_1)}{\rho_1(\rho_0 - \rho_2)}$$

If suitable numerical values are substituted in this expression for the natural and regenerated fibres their relative crystallinity turns out to be approximately that given by other physical techniques. That it is not the same should not be surprising because what the density method measures as crystalline depends on many factors and is difficult to define precisely.

The moisture regain of cellulose has also been used[12] to determin its crystallinity and accessibility to water. The assumption in thi work is that the nature of the sorbing material in different types o cellulose is the same and, therefore, any difference in moistur regain must be ascribed merely to difference in amount of sorbin substance. Hermans postulated[17] that, since the disordered region are where the water is absorbed, the moisture regain, particularl in the range 20–70 per cent relative humidity, should be a measur of the amount of this type of material present. On this basis, the quantity of amorphous substance in natural, mercerized, and regenerated celluloses should be in the proportion 1 : 1·5 : 2. Other workers[18,19] have questioned the basis of this postulate on the grounds that X-ray evidence indicates that water can penetrate the crystalline regions of cellulose II to form true hydrates, but cannot do so with the cellulose I crystalline regions. If this is so, then, for a given amount of amorphous material, a cellulose possessing the crystalline form of cellulose II should have a higher moisture regain than one containing the cellulose I crystalline form. However, the question of whether water can in fact penetrate the crystalline regions of cellulose II has been disputed by many independent investigators,[20–22] and recent infra-red[8] and X-ray evidence[23] is against the idea.

Present ideas regarding moisture uptake are that the accessible hydroxyl groups and other potential sites contributing to the sorption may be in the disordered regions or partially disordered regions, and on the surface of the crystallites themselves. Thus for two celluloses of the same crystallinity, but different crystallite size, one would expect the sample with the smaller crystallites to exhibit the higher moisture regain. It follows then that there might be at least an approximate correlation between the moisture regain of a cellulose and the percentage of OH groups which are accessible and in an amorphous environment—this includes, of course, the OH groups that protrude from the surface of the crystallites. A measure of the percentage of these OH groups in various types o cellulose has been obtained[8] using the infra-red method described earlier, and Valentine[24] has shown that if these are plotted against the sorption ratio (i.e. the moisture regain of any cellulose relative to that of cotton under the same conditions) of the corresponding celluloses a straight line is obtained. Furthermore, if this line is extrapolated back it passes through the origin as indeed it should if the correlation is correct. A plot of these results is shown in Figure 3. Thus this method does seem to provide an easy and convenient way of estimating the disordered regions of a cellulose sample.



A: Cotton micelles; B: Fortisan micelles;
C: Viscose rayon; D: Mercerized viscose rayon

Figure 3. The relation between sorption ratio (S.R.) and the fraction of amorphous OH groups (F_{AM}) *for various types of cellulose*[24]

Chemical Methods for Accessibility and Crystallinity

Several investigators[25,26] have made attempts to determine the accessibility of cellulose in non-swelling media, and as may be expected, each has reported a very low value. In each case the reagent used was unable to penetrate the sample to any extent and, hence, it is apparent that this method reflects gross structural variations rather than the finer details of the structure. Of the chemical methods for determining accessibility using a swelling media, mention may be made of the hydrolysis–oxidation method pioneered by Nickerson.[2,3] The amount of cellulose hydrolysed to glucose in a given time was determined by comparing the carbon dioxide liberated from the sample with that liberated from a known amount of glucose treated in the same way. A plot of these results reveals that the rate of hydrolysis, which is initially rapid, gradually slows down to a more or less constant rate associated with the more highly ordered crystalline regions. By extrapolating the curve back to zero time, a rough measure could be obtained of the amount of amorphous cellulose in the sample.

This kinetic method, sometimes slightly modified, has been applied by several investigators[27–31] and the rates of various reactions have been studied usually with the assumption that the chemical reaction does not materially alter the physical structure of the material being investigated. Lovell and Goldschmid[30] used the Nickerson reagent (hydrochloric acid in the presence of ferric chloride) to characterize various celluloses, but instead of estimating the hydrolysis-liberated glucose by measuring the carbon dioxide evolution, they assumed that the glucose formed on hydrolysis may be determined by the actual loss in weight of the sample.

One of the main objections that has been raised against hydrolytic methods in general is that, under the severe acid conditions necessary, there is likely to be an increase in crystallinity during hydrolysis. This has in fact been shown[32] to be true in the case of regenerated celluloses, but for natural celluloses the results[32,33] are as yet inconclusive. A second criticism of the hydrolytic method is that, in many cases, the initial weight loss corresponding to the accessible regions is quite small, and the distinction between the accessible fraction and the more ordered regions can only be achieved by arbitrary extrapolation. However, it has been shown[31,34] that whereas the initial weight loss is small, the fall in the degree of polymerization is usually great, and the investigation of the chain breakdown during hydrolysis provides a sensitive method for determining accessibility. It is thought that the advantages of this over the Nickerson method lie in the fact that it is not necessary to study high degrees of degradation and hence the accessibility can be evaluated before much recrystallization occurs. In addition, since this technique employs a sensitive method of measuring the degradation, very mild hydrolytic conditions, e.g. 0·1N sulphuric acid at 50°, can be used.[34]

The sort of results[2,3,27–31,34,35] that have been obtained by the method of acid hydrolysis for various types of cellulose are shown in Table 3. It will be noted that the values obtained are low compared to the deuterium-oxide exchange method, and the reason for this is no doubt connected with the recrystallization that takes place during the reaction. Until more is known about what is actually happening during the hydrolysis a great deal of significance should not be attached to the absolute values obtained. Nevertheless, the technique is useful as a rapid and accurate method for the comparison of different celluloses particularly when the crystallinity is high, for under these circumstances physical techniques such as X-ray diffraction are not sensitive to small variations in the amount of ordered and disordered material.

The class of chemical methods which rely upon reagents which react with a limited percentage of the total number of OH groups present is not usually subject to the criticism of recrystallization during the reaction. The isotopic exchange reaction with heavy water and the formic acid esterification[36,37] are typical methods in this class.

Champetier and Viallard[10] studied the reaction between cellulose and liquid deuterium oxide by measuring the density of the heavy water at various times during the deuteration, and thus obtained an estimate of the amount of water liberated. They reported that filter paper and linter cellulose were completely accessible to deuterium oxide, and the exchange reaction was completed in 36 hr at 30°. Frilette, Hanle, and Mark[4], however, using a similar method, obtained quite different results. They found that the reaction was initially rapid but after 4 hr had become so slow that the exchange was very little different from that after 1 week. These authors suggested that the fraction that had not reacted after 4 hr should be identified as crystalline material. The reaction has also been studied spectroscopically by Rowen and Plyler,[38] who reported that the exchange was actually much less than the value obtained by Mark. However, Almin,[39] and Mann and Marrinan,[8,9] also using an infra-red method, support the conclusions of Mark and suggest that the low exchange found by Rowen and Plyler was due to re-hydrogenation of the samples before the infra-red measurements were carried out. Some of the results obtained[4] using densitometric measurements to follow the deuterium exchange reaction are shown in Table 3.

Table 3: Accessibility of cellulose

Material	Percentage accessibility			
	By deuterium exchange[4]	By formylation[36,37]		By acid hydrolysis
		(a)	(b)	
Cotton	41	28	16	5–13
Bacterial cellulose	—	—	—	3
Mercerized cotton	—	52	28	11–32
Fiber G	67	65	—	19–30
Textile rayon	68	—	54	22–32
High-tenacity rayon	86	77	—	25–38

(a) 98 per cent formic acid, 15 hr at 55°.
(b) 90 per cent formic acid, 12 hr at 30°.

Typical accessibility values determined by formic acid esterific tion are also shown in this table, and these illustrate that with th method, the values obtained depend markedly on the conditio used. Unlike the hydrolysis method, recrystallization during th exchange reaction and during the formylation does not occur any great extent. In the case of the latter, the reason for the inhib tion of the recrystallization tendency has been shown by Baker to be due to the partial substitution of the cellulose.

THE CRYSTALLINE FORMS OF CELLULOSE

In the chapter on the crystal structure of cellulose it was pointed ou that there are four different crystalline modifications of cellulos which have been characterized by X-ray diffraction methods an which are now generally referred to as cellulose I, II, III, and IV Recent estimates of the unit cell dimensions of these four crystallin forms are shown in Table 4; for cellulose I and II, these are slightl different from those given in the preceding chapter.

Table 4: Unit cell dimensions[41]

Material	Type of cell	Axis			
		a	b	c	β
Cellulose I ...	Monoclinic ...	8·2 Å	10·3 Å	7·9 Å	83°
Cellulose II ...	Monoclinic ...	8·0 Å	10·3 Å	9·1 Å	62°
Cellulose III ...	Monoclinic (Hexagonal) ...	8·6 Å	10·3 Å	8·6 Å	60°
Cellulose IV ...	Orthorhombic ...	8·07 Å	10·3 Å	7·9 Å	90°

It will be noted from this table that there is a marked similarit between the unit cells of cellulose I and IV and between cellulose II and III and Howsmon and Sisson have suggested[42] that cellulos III and IV can be regarded as disordered forms of cellulose II and respectively.

Recently these crystalline modifications have been studied[43] b infra-red spectroscopy and some interesting results obtained. Th technique employed in this method of studying crystalline region utilizes the deuterium isotopic exchange reaction described previ ously whereby heavy water vapour is allowed to react with th sample of cellulose being examined and, thereby, cause an exchang to take place between the deuterium atoms of the heavy water an the labile hydrogen atoms of the amorphous regions of the sample

If the infra-red spectrum is now examined in the wavelength region where the hydrogen-bonded OH groups absorb, then, since these groups are only present in the crystalline regions, these may be studied without interference from absorptions arising from the amorphous material present. The results obtained by this method will be treated separately under the heading of each of the above crystalline forms.

Cellulose I

Various samples of cellulose which were known to possess the cellulose I crystalline modification were studied with particular reference to their hydrogen-bonded OH groups. It was found that they gave two types of spectra and these are shown in Figure 4.

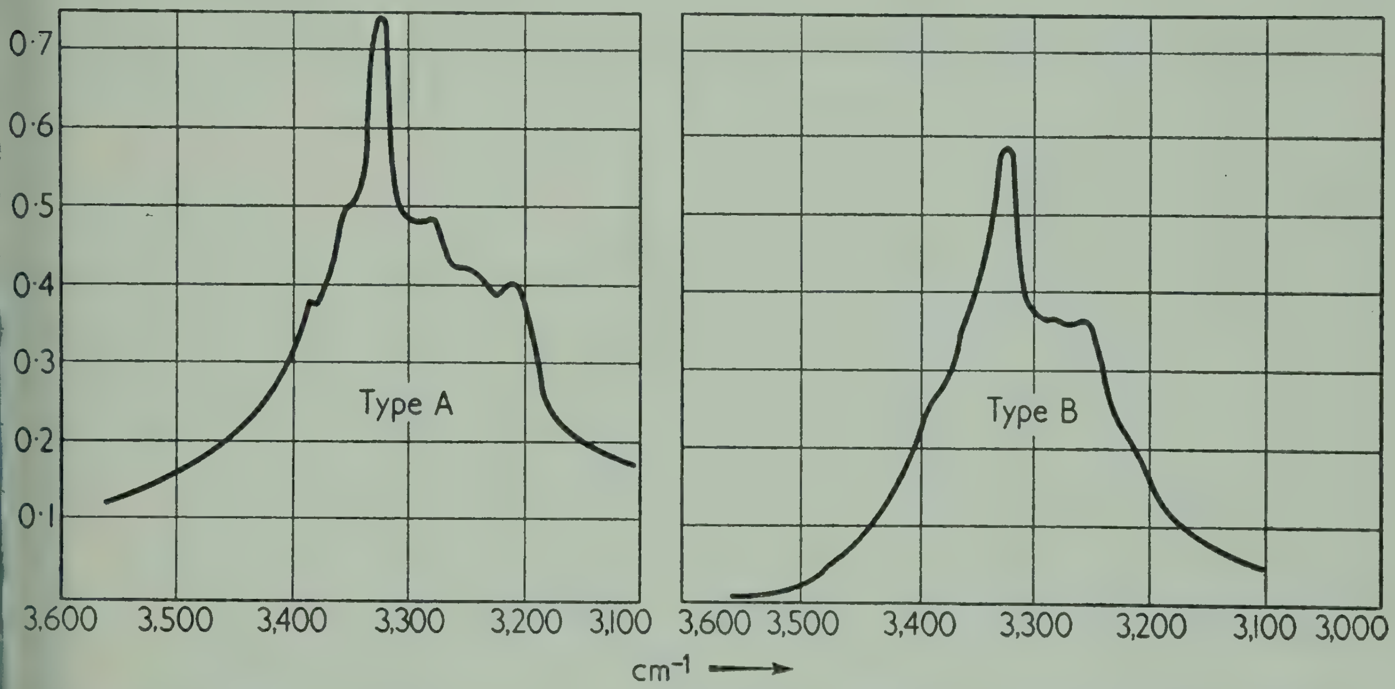


Figure 4. Spectra of cellulose I. Upper: Valonia ventricosa *cellulose; lower: Tunicin cellulose; Both at the temperature of liquid nitrogen*

The only forms of cellulose which have been found to give type A spectra are celluloses formed by bacteria and the alga *Valonia ventricosa*, while all other forms of cellulose I which have been studied, including the plant celluloses and regenerated cellulose I, gave type B spectra. It is also worth noting that it is possible to modify the material with a type A spectrum so that it gives a type B spectrum, but it has not been found possible to do the reverse. For example, bacterial cellulose (type A spectrum) may be converted into cellulose III or cellulose triacetate I, and when cellulose I is regenerated from these products the resulting crystalline form has a type B spectrum. It has been shown that the difference in the two types of spectra is unlikely to be due to a nitrogen-containing impurity but rather implies a difference in the molecular packing in the crystals of the two types of material. This could be due to different crystal structure in the two cases or to a difference in th degree of perfection of crystals having the same basic molecula arrangement. However, at the present time, it is not possible t be certain what the reasons are for this difference in spectra.

Cellulose II

No difference was found in the hydrogen bonding of the crystal line regions of mercerized and regenerated celluloses. Thei spectrum is identical to that shown for viscose in Figure 2. Althoug Wellard[41] has reported that the unit cell angle β is slightly greate for mercerized cellulose than for most regenerated celluloses, thi would not necessarily lead to a change in the hydrogen bonding For example, if the hydrogen bonding is of the layer type in cellulos II a difference in the angle β could be due to a difference in the arrangement of the hydrogen-bonded layers relative to one another

Cellulose III

Although samples of cellulose III prepared from cellulose I and I respectively appear to have identical unit-cell dimensions,[41] a close examination of the X-ray diffraction photographs reveals that ther are differences in the relative intensities of various reflections. Th infra-red spectra of these two types of cellulose III are shown i Figure 5, and they clearly indicate that the two cellulose III's ar

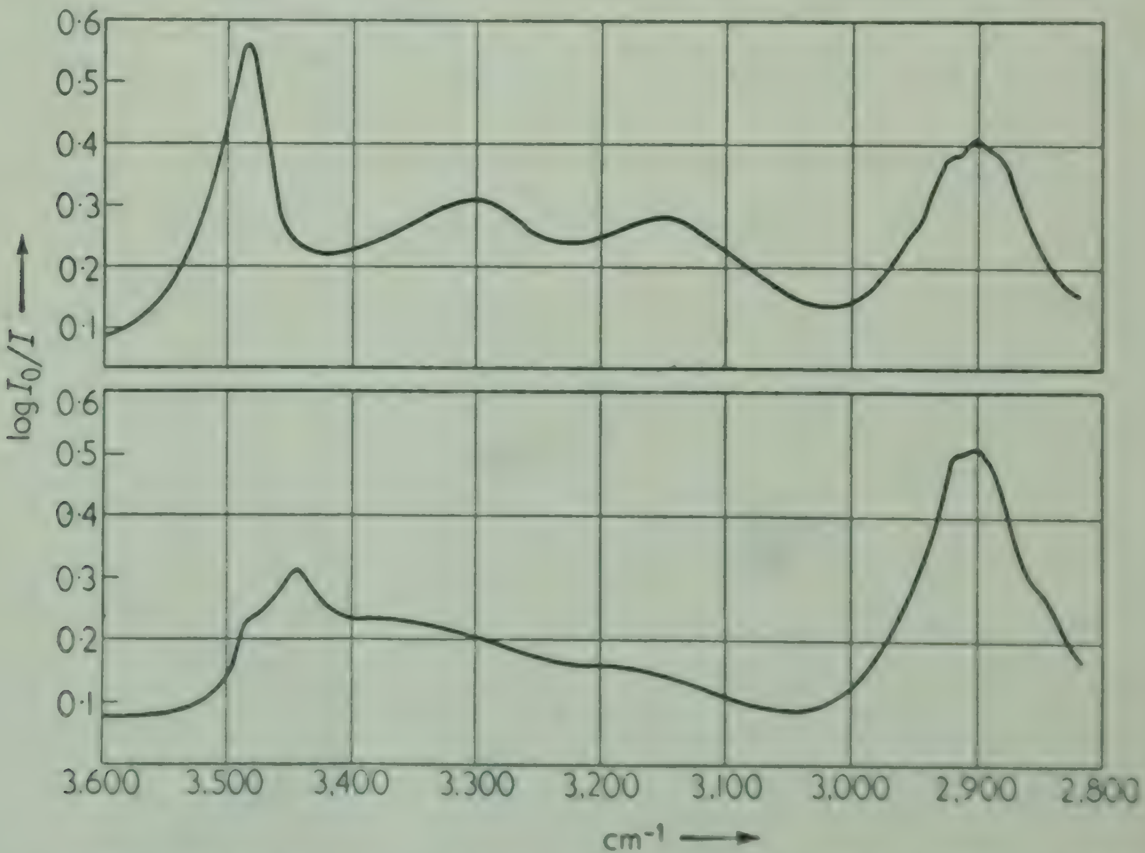


Figure 5. Spectra of cellulose III. Upper: III_I prepared from I; lower: III_{II} from II

different. This view is supported by their behaviour towards water. When treated with water at room temperature, cellulose III prepared from cellulose I slowly reverts to cellulose I. In boiling water, the reconversion is faster and is complete after about 10 hr. On the other hand, cellulose III prepared from cellulose II reverts to cellulose II when treated with water, but this is much more difficult to accomplish than with cellulose III from I. This behaviour towards water, which has been observed previously by X-ray diffraction methods,[44,45] coupled with the difference in the infra-red spectra suggests that the two materials should be regarded as different crystalline modifications. It has been suggested[43] that they be designated III_I and III_{II} to indicate their origin.

The spectrum of cellulose III_I makes it clear that the suggestion by Howsmon and Sisson that cellulose III is a disordered form of cellulose II, because of the similarity of their unit cells, cannot be supported.

Cellulose IV

The infra-red spectra of cellulose IV prepared from cellulose II, and from cellulose I by the route I → III → IV are shown in Figure 6. The method used for preparing this crystal form from cellulose I gave a product containing at least 60 per cent of cellulose IV.

As is seen, different spectra are obtained from the two materials,

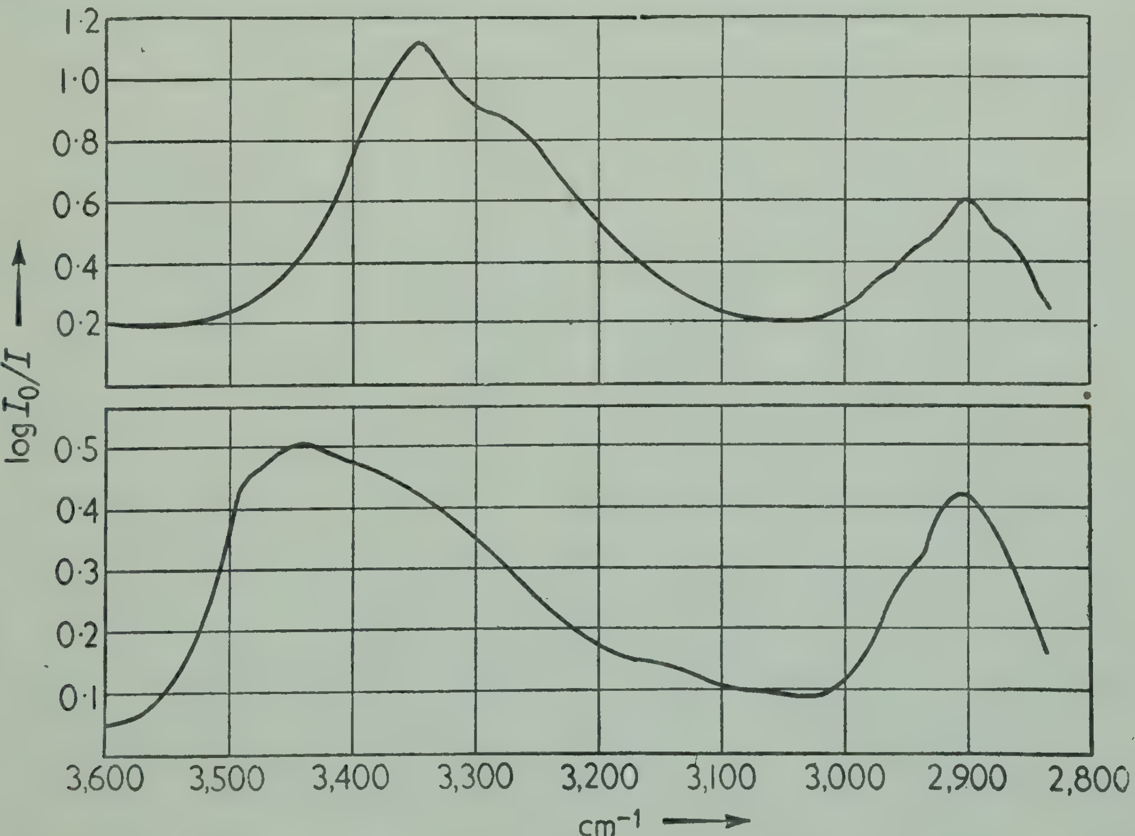


Figure 6. Spectra of cellulose IV. Upper: IV_I prepared from I; lower: IV_{II} prepared from II

which can be designated IV_I and IV_{II}. Thus cellulose IV_I and IV_I must be regarded as being different despite the fact that their uni cells are the same. It will be noted however, that the spectrum o IV_I resembles that of cellulose I. This is in harmony with the X-ra data on the unit cell dimensions. On the other hand the spectru of IV_{II} resembles in general appearance that of cellulose II althoug there are considerable differences in the relative intensities of th bands, and provides no support for the suggestion that it is a disordered form of cellulose I.

Conclusion

This recent infra-red work shows the value of spectroscopy in examining and elucidating fine details of crystal structure. This applies particularly to polymers like cellulose, containing chemical groups which form hydrogen bonds, as these are particularly sensitive to changes in crystal form. The results obtained by this method make it clear that cellulose I and II give different forms of cellulose III and IV. In addition, the behaviour of III_I and III_{II} towards water suggests that these materials retain a ' memory ' of the crystalline form from which they were made. In particular, III_I reverts to cellulose I in water despite the fact that cellulose II seems to be the stable form in the presence of water.[46] The results suggest, therefore, that cellulose I and II contain some structural feature that makes it difficult for them to be converted into the same form of III or IV. This difference between I and II will be discussed in more detail in a later section.

HYDROGEN BONDING IN THE CRYSTALLINE REGIONS

Despite extensive studies of the various polymorphic forms little is known about the details of molecular arrangement within the crystalline regions. One of the reasons for this is that the X-ray diagram of cellulose contains too few reflections to allow definite atomic configurations to be obtained from X-ray evidence alone. The structures which have been proposed[47,48] for cellulose in the past have been based partly upon the available X-ray data and partly upon stereochemical evidence. One difficulty in using the latter kind of evidence is that the detailed structure of the closely related oligosaccharides has not yet been worked out, and thus the form of the cellobiose repeating unit in cellulose is not precisely known.

It is apparent that information provided by other physical techniques, which can be used in conjunction with the X-ray data, would be of great help in providing a satisfactory solution to this problem. In this respect, the importance of a technique for studying the hydrogen bonds in cellulose cannot be over-emphasized for it is largely the forces associated with these bonds, coupled of course with the size and shape of the molecules themselves, which determine the arrangement and packing of the molecules in the ordered regions.

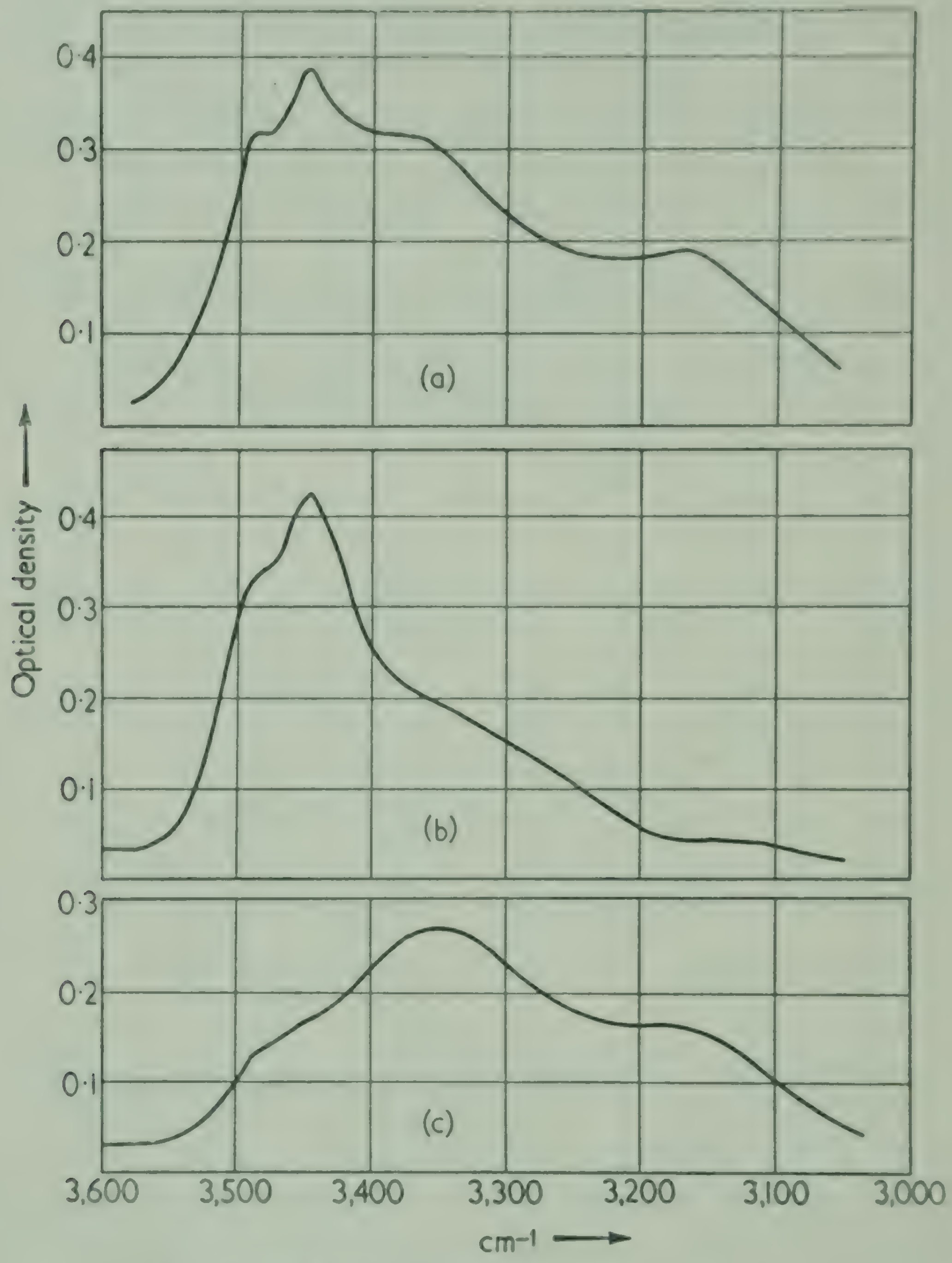
In a recent paper, Mann and Marrinan,[43] using polarized infra-red spectroscopy have studied these hydrogen bonds in various crystal modifications of cellulose. Although this evidence cannot give the precise atomic configurations in the crystal it does enable many trial structures to be eliminated without recourse to the time-consuming calculations of X-ray intensities. The studies that have been made on the various crystal modifications will be presented under their separate headings.

Cellulose II

The infra-red spectrum of the hydrogen bonds in viscose taken using plane-polarized radiation is shown in Figure 7.

Although it is not possible, without the use of a single crystal of cellulose, to calculate from the observed dichroic ratios the exact angle that the hydrogen bonds make with the chain direction, it is apparent that the hydrogen bonds responsible for the two sharp bands at 3,484 cm^{-1} and 3,444 cm^{-1} make angles appreciably smaller than the 54° 44′ required[50] for a dichroic ratio of unity. The remaining bands shown in this spectrum are due to hydrogen-bonded OH groups which make angles with the chain direction greater than 54° 44′. This type of behaviour is shown by all three crystalline forms which have been studied by infra-red spectroscopy. Molecular models show that only OH groups which form intra-molecular hydrogen bonds linking successive glucose units in the cellulose chain can make a small angle with the chain direction. The possible bonds are O_3—H . . . . O_5', and O_2—H . . . . O_6', or O_2 . . . . H—O_6', where primed atoms are in an adjacent residue in the same chain.

Although only four bands can be seen in the spectrum of viscose shown, the absorption near 3,350 cm^{-1} in fact contains two bands[43] making in all three distinguishable hydrogen bonds which make a large angle with the molecular chain direction. In addition it has been shown[43] from the similarity of the spectra of cellulose II and III_{II} that the hydrogen bonding in cellulose II probably involves bonding between the centre and corner chains of the unit cell. The arrangement of the hydrogen bonds which has been found[49] to fit the stereochemical and spectroscopic data for cellulose II best is shown diagrammatically in Figure 8. This has all the OH groups hydrogen-bonded with the intramolecular bonding only between residues in the (10$\bar{1}$) plane (β-acute convention). The central chain



A: Unpolarized radiation;
B: Electric vector parallel to chain direction;
C: Electric vector perpendicular to chain direction

Figure 7. Polarized spectra of cellulose II

is reversed with respect to the corner chain and shifted by about 0·28 of the unit cell length along the chain direction. Both molecular chains have a two-fold screw axis and differ only in the positions of their O_6 atoms. In Figure 8, the cellobiose repeating unit has been drawn in for clarity but it does not represent its actual form. Each glucose residue is in the chair configuration and they are inclined to each other, both in the plane of the figure and perpendicular to this plane. This arrangement is quite distinct from the form of the cellulose chain proposed in the Meyer and Misch[47] structure, and in fact it is interesting to note that it is very similar to the form of the molecular chain recently determined[51] independently for α-chitin—a closely related polymer, and to that suggested by Petitpas and Mering[52] for cellulose from recent X-ray data.

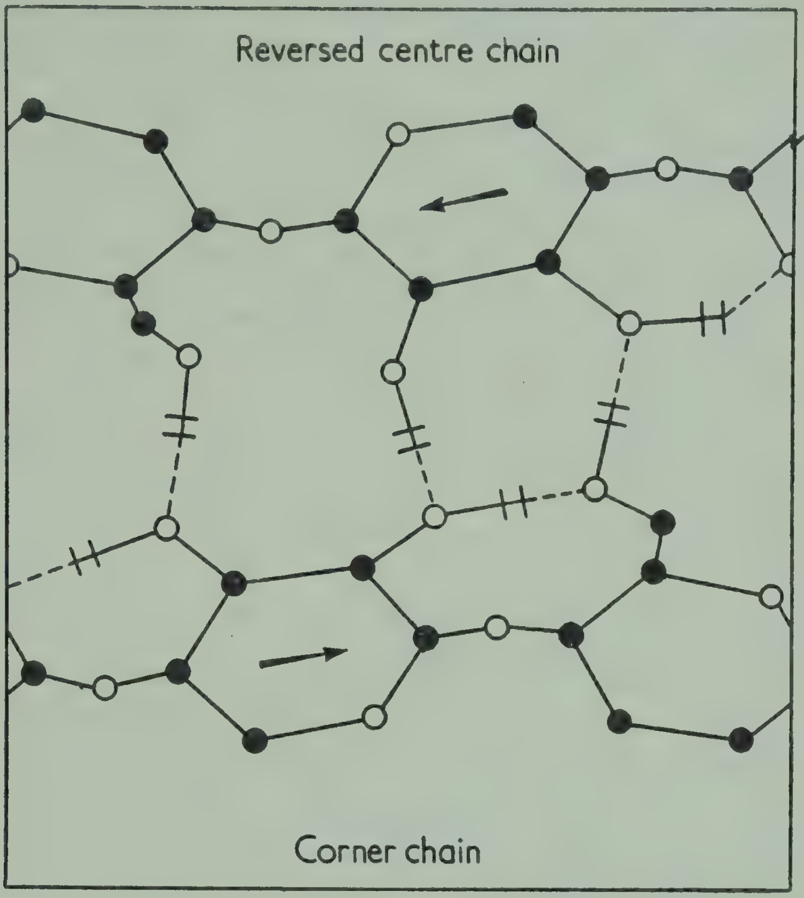


Figure 8. The suggested arrangement[49] of the hydrogen bonds in cellulose II

Cellulose III_I

Cellulose III_I shows only three infra-red absorption bands (see Figure 5) which are due to the stretching vibration of hydrogen-bonded OH groups. The band at 3,484 cm^{-1} exhibits parallel dichroism,[49] is sharp, and is almost certainly due to only one type of intramolecular hydrogen bond. It is possible to show from symmetry considerations that there are two ways in which a structure based on cellobiose residues having a two-fold screw symmetry can have only three OH groups which would be distinguishable in the infra-red. Both cases require the asymmetric unit of the structure, which consists of a glucose unit of the corner chain and a glucose unit of the centre chain, to be related by a non-crystallographic symmetry operation. These are discussed in detail in the original paper.[49] Using this spectroscopic evidence, Mann and Marrinan have proposed two possible hydrogen-bonding systems for cellulose

III_I. These are shown in Figure 9. As in the arrangement proposed for cellulose II, the OH groups in the structure proposed for cellulose III_I are all hydrogen-bonded with intermolecular bonding only in the $(10\bar{1})$ plane.

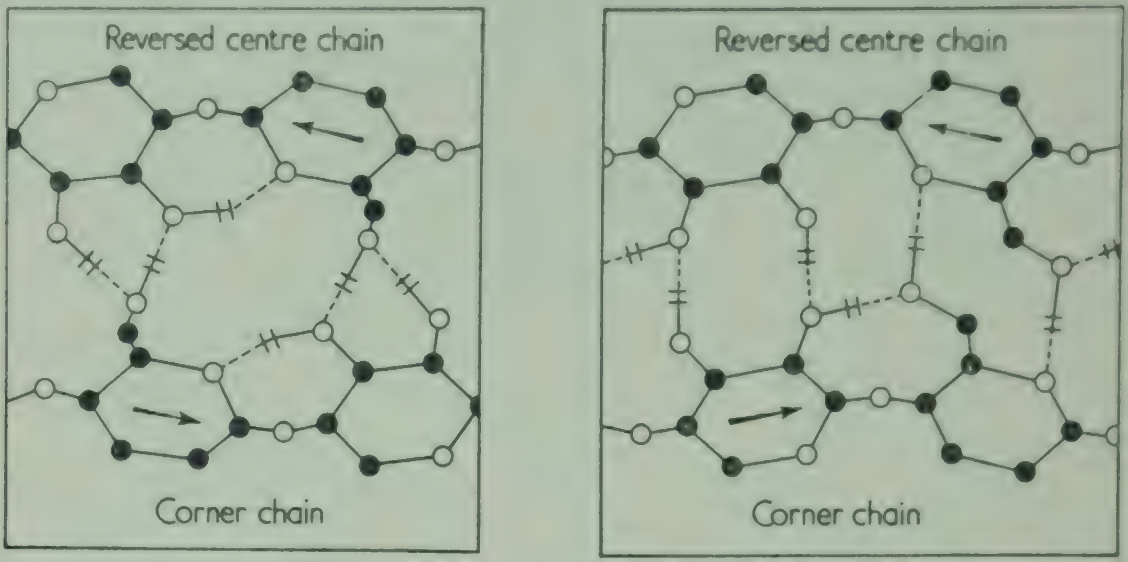


Figure 9. Two possible arrangements[49] *of the hydrogen bonds in cellulose* III_I

Cellulose I

The infra-red evidence on the structure of cellulose I is not so precise as that for cellulose II and III_I. This is partly due to the fact that two different spectra have been observed for different cellulose I samples. Until it becomes possible to understand more fully the reason for these differences in the spectra, caution should be observed in proposing a structure based on infra-red and stereochemical evidence alone. However, the difference in stretching frequency between the hydrogen bonds which are approximately parallel to the molecular chain in cellulose II and III_I on the one hand, and those of cellulose I on the other,[49] is appreciable and suggests that the molecular form of the cellobiose unit may be slightly different in cellulose I from the other two modifications.

As was pointed out in an earlier section, there is a structural difference between cellulose I and cellulose II which makes it difficult or impossible for them to be converted directly into the same form of cellulose III or IV and, furthermore, this structural difference persists in the new modifications as shown by their behaviour towards water. It has been shown[43] that Hermans' statement[53] that cellulose I is obtained when cellulose triacetate is hydrolysed at 100° by 2N ammonia is incorrect. All the other ways[54] reported for converting cellulose II into I use methods which would undoubtedly lead to cellulose IV. Thus the only known way in which a transformation can be effected between I and II is by the use of powerful swelling agents as in mercerization

These swelling agents penetrate the crystalline regions and must give considerable mobility to the cellulose chains. It seems possible, therefore, that the structural difference between cellulose I and II referred to above resides in a difference in the relation of the centre and corner chain of the unit cell in the two cases. This difference could, for example, be a difference in the shift of the central chain along the b axis of the unit cell or it could be a difference in the polarity of the central chain with respect to the corner chains, i.e. parallel chains in one crystal form and antiparallel in the other. This latter possibility would imply that the process of mercerization involves the disruption of crystallites and the formation of new ones. However, it is not possible at present to be certain what the structural difference is between cellulose I and II which causes these forms to behave differently.

THE NATURE OF THE AMORPHOUS REGIONS

The so-called ' amorphous ' material present in polymers is usually taken to mean all the material which is not actually present in a crystalline form as defined by a physical technique such as X-ray diffraction. These amorphous regions are often regarded as consisting of material of various degrees of order up to, but not including, that exhibited by the crystalline regions. Usually more than half the material is composed of this sort. Hence we see the prime importance of these regions, not only from the point of view of the chemical reactivity of cellulose, but also because the mechanical properties may well be expected to depend a great deal on them.

It has been apparent for a number of years that the measurement of the crystalline–amorphous ratio of a cellulose sample is by no means sufficient to characterize, or to differentiate completely between, different celluloses. In fact, this type of measurement may be quite misleading in that it under-emphasizes the vast difference between various types of cellulose. Without doubt, these differences cannot be accounted for solely by the amount or type of crystalline form present and must be attributed, at least in part, to differences in the nature of the amorphous regions.

In cellulose it is well known that the polymer chains are very much longer than the individual crystallites and hence one chain may pass through many crystallites and hence through many different amorphous regions. Since the mechanical properties often depend upon the behaviour of segments of the polymer chain containing many monomer units, it is also important to determine how these amorphous regions are linked together.

Unfortunately, due to the lack of order in the amorphous regions many of the common physical methods do not lend themselves to the study of the nature and arrangement of the polymer chains, and most of the information available has been obtained from chemical methods

Cellulose can be obtained with a low degree of crystallinity by evaporation of ammonia[55] or a primary alkylamine[43,56,57] from the ammonia- or amine-cellulose complex, or by dry grinding of cellulose in a vibratory ball mill.[58] These materials, when examined by X-ray diffraction, give a very broad ring indicating a structure similar to that found in a low molecular weight liquid. However, these highly amorphous celluloses are very unstable towards water and crystallize readily on wetting. Thus it is unsafe to assume that there is anything other than a formal connection between these celluloses and the structure of the amorphous regions in a polycrystalline cellulose material.

The idea that there is a gradual rather than a sharp transition from order to disorder in cellulose has been frequently expressed, and several attempts have been made to show that such a polyphase structure does in fact exist. Howsmon and Sisson[59] have defined the degree of order in a small volume element of the amorphous regions in terms of the concentration of hydrogen-bonded hydroxyls. However, infra-red evidence[8] has shown that in the amorphous regions of many different celluloses all but a very small percentage of the hydroxyls are hydrogen-bonded and, furthermore, there appears to be very little difference between the nature of the hydrogen bonds in the amorphous regions in such widely different forms of cellulose as bacterial and viscose. Thus, this definition does not appear to be a satisfactory one for characterizing these regions.

Confirmation of the fact that there are differences in the degree of order of various celluloses is shown by the experimental approach[60,61] of swelling samples of cellulose in a given solvent, in which the solvent power can be progressively increased by increasing either its concentration or its temperature, and subsequently determining such properties as the degree of crystallinity,[62] the moisture regain,[46,63,64] or levelling-off degree of polymerization.[61,64]

It is generally supposed that at low concentrations the solvent penetrates only the low-order amorphous regions but that the penetration increases gradually with increasing concentration of solvent. After removal of the solvent the order in these regions is lower than in the original sample and hence accounts for the change in the above properties. The type of result obtained by this method is illustrated in Table 5.

As shown in Table 5 and as stated by Jörgensen and Ribi[64] the pulp with the lower degree of polymerization seemed to be more difficult to mercerize partially than the material of higher degree of polymerization. They say that this indicates that the transition from cellulose I to cellulose II is dependent also upon the intercrystalline network, in that long cellulose chains extending through and connecting the crystallites facilitate the transformation to the cellulose II lattice. If it is assumed that the slope of a graph of any one of these properties plotted against solvent concentration is proportional to the quantity of material being modified in a given concentration interval then the 'lateral order distribution' can be estimated[65] to a first approximation. It is apparent that the shape of the distribution determined by this method will vary with the property used, for usually the moisture regain and the intrinsic viscosity change at a lower concentration than is required for modification of the crystalline lattice from cellulose I to cellulose II as measured by X-ray diffraction. However, this method is useful in giving a qualitative estimate of the distribution curve for natural celluloses, but difficulties are experienced with mercerized or regenerated celluloses either because their properties are only slightly affected[65] or the situation is complicated by the effects of recrystallization.

Table 5: Limit DP, moisture regain, and X-ray data for wood pulps swelled in alkali solutions of varying concentrations[64]

Swelling solution (percentage weight sodium hydroxide at 3°)	Pulp DP = 1,700			Pulp DP = 1,300		
	Moisture regain (per cent)	Limit DP	X-ray data	Moisture regain (per cent)	Limit DP	X-ray data
0	6·5	240	Cellulose I	6·6	225	Cellulose I
5·5	6·6	140	Cellulose I	7·7	115	Cellulose I
6·0	8·3	95	Cellulose I and II	8·1	75	Cellulose I
7·0	8·4	80	Cellulose I and II	8·3	75	Cellulose I and II
7·5	8·7	75	Cellulose I and II	8·7	65	Cellulose I and II
8·0	8·8	55	Cellulose II	8·8	55	Cellulose II

Several other methods have been used in the quest for information about the nature of these disordered regions but as yet none is entirely satisfactory. As a consequence probably less is known about the amorphous regions in cellulose and the way they differ betwee the various celluloses than about any other facet of cellulose fin structure.

ORIENTATION OF CRYSTALLINE AND AMORPHOUS REGIONS

The term ' orientation ' usually refers to the alignment of th individual molecules relative to one another and to a given directio or surface of the sample. In a non-crystalline fibre, measurements o orientation relate to the alignment of the axis of the polymer molecules with the axis of the fibre. Even if the molecules in such a sample have a very high orientation, they will not exhibit the properties of crystalline regions unless a reasonable three-dimensional order is also present. In the ideal case, it is desirable to measure the distribution of orientations—i.e. to determine the fraction of molecules which lie at any particular angle to a chosen axis. This is a difficult task to accomplish, however, and it is more usual to see figures quoted for the average orientation of the molecules.

In crystalline specimens, the situation is in some ways more complex, for certain crystallographic planes may tend to set themselves parallel to the sample surface, or the crystals tilt with respect to the fibre axis in special crystallographic directions. Certainly the molecular chain orientation in the crystalline regions may be very different, both in distribution and on the average, from the rest of the molecules making up the disordered regions. Because of this lack of correlation, the extent to which it is possible to characterize the orientation in a specimen depends on the experimental methods available for distinguishing between the orientation in the crystalline and the amorphous regions and for measuring the distribution. Several experimental methods are available, each of which gives a limited amount of information, and in this section these methods will be discussed.

X-ray Diffraction

Of the various experimental methods which have been employed for measuring orientation, the X-ray method has been the most widely used and is in many ways the least controversial. It distinguishes most clearly between the orientation of the amorphous and crystalline regions and, most important, it is the only one which is capable of giving a distribution of orientations.

The X-ray diffraction pattern of the crystalline regions of a polymer is characterized by discrete reflections, usually in the form of short arcs. The intensity distribution along the length of these arcs may be interpreted in terms of the distribution of certain crystallographic plane orientations. It may be found that different reflections have not the same distribution of intensity, indicating that not all the crystallographic planes have the same distribution of orientation. In other words, the crystals have been orientated so that certain planes are more completely aligned than others.

If it is desired to compare the X-ray results with other techniques for obtaining orientation (all of which give an average chain orientation), it is necessary to express the X-ray results in a similar way, rather than in terms of crystal-plane orientations. In general, these two are not the same because a particular crystal-plane orientation can be compatible with a range of chain orientations. However, if measurements are made on more than one equatorial arc then the molecular chain orientation may be determined uniquely. Hermans[66] has developed a method for carrying out this conversion for cellulose fibres and has used as an index of the orientation an orientation factor f_x where $f_x = 1 - \frac{3}{2} \sin^2\bar{\alpha}$ and $\bar{\alpha}$ is the average angle of orientation of the crystallites. Some of the results that he has obtained are shown in Table 6.

Table 6: Average angle of longitudinal orientation α and orientation factor f_x of the crystalline regions of various fibres as derived from the X-ray diagram[66]

Material	α	f_x
Ramie	7° 6′	0·97
Ramie mercerized without tension ...	15° 0′	0·90
Ditto re-oriented	6° 3′	0·98
Viscose rayon without stretch	22° 4′	0·79
Viscose rayon with 120 per cent stretch ...	14° 3′	0·91

For some purposes, however, it is sufficiently accurate to disregard the possibility of selective orientation of the crystallite planes, and to estimate the orientation from the angular spread of a particular equatorial reflection. This may be done conveniently by using, as an index, the angular length of the arc between the two positions of half-maximum intensity. This assessment is often used for cellulose fibres, since selective orientation effects of the kind mentioned above are not often present. If films of cellulose are employed, however, these effects are more likely to be present and indeed films are often specially prepared for observing these phenomena.

Other types of characteristic orientation are sometimes observed in cellulose fibres. Probably the best known is in cotton where the crystallites are found to be oriented at a small angle to the fibr axis corresponding to the helical configuration of the fibrils.

Amorphous material in polymers gives a diffuse X-ray scatterin pattern. If the amorphous material is disoriented, this pattern take the form of a complete ring, whose width is a measure of the distribution of the spacing between the molecular chains. If the amorphou material is oriented, then the distribution of intensity along th ring is no longer uniform; its variation with angle to the equator i some indication of the distribution of molecular orientations in th amorphous regions. The exact relationship is, however, comple and only rough conclusions may be drawn about the orientation o these amorphous chains from the X-ray pattern. Because of this very little work has been published on this aspect.

Although much good work has been done by the X-ray method and our knowledge of the molecular orientation of polymers i largely due to this technique, it cannot be described as a ver sensitive method quantitatively. It is simplest and most reliabl when the amorphous scattering shows a definite band, which ca be resolved from the crystalline reflections. Often, however, th broad reflections of the amorphous material overlap the sharpe crystalline ones and reduce the accuracy of the information which can be obtained. This, unfortunately, is the case with cellulose.

When the orientation is poor, the X-ray reflections (on flat films) tend to complete circles with a small variation of intensity along the circle. Under these conditions, the X-ray method is not ver sensitive and the determination of the orientation by the optica birefringence method is often preferred.

Optical Birefringence

The polymer molecules in both the amorphous and crystallin regions of a fibre usually show a tendency to orient their long axe parallel to the fibre axis but show no preferential orientation o either of the other two perpendicular axes. The effect of this lac of order in the lateral direction leads to an averaging of the optica properties perpendicular to the fibre axis and thus the specime behaves optically like a uniaxial crystal. Therefore the refractiv indices for light polarized parallel and perpendicular to the fibr axis will in general be different. This difference is called the birefringence and may be determined by measuring the difference i optical path between these two directions. This is usually done b interposing in the light path a compensator of the Babinet or Berek type which can be adjusted to make the optical path of the tw polarized beams equal. The expression for the optical path differ ence is $(n_1-n_2)t$ where n_1 and n_2 are the two refractive indices and t is the thickness of the specimen. Thus from the measured path difference, and a knowledge of the thickness, the birefringence may be calculated.

An alternative method of measuring the birefringence is to determine the two refractive indices separately. This can be done by immersing the fibre in a liquid of approximately the same refractive index as the one it is desired to measure and then changing the refractive index of the immersion liquid either by altering its temperature, or by changing the wavelength of the light used until the edge of the fibre is no longer visible. This method is known as the Becke line method because of the bright fringe or line of light that is observed near the edge of the fibre when the refractive indices are unequal. When the microscope objective is raised the bright fringe moves towards the medium of higher refractive index, thus indicating whether the refractive index of the immersion liquid has to be increased or decreased to match that of the fibre.

As distinct from the compensator method which measures the average birefringence through the fibre the Becke line method is usually assumed to measure the refractive index near the surface of the fibre. Faust[67] has recently discussed the application of the Becke line to fibres and concluded that the accuracy of the index measurement is a function of the fibre shape and dimensions and of the degree of defocusing of the microscope. He also concludes that in the case of fibres with very thin skins the refractive index values that are obtained are not necessarily those of the surface regions.

If it is desired to measure the refractive index of the skin then the degree of defocusing must be less than a certain value (4 μ in the case of a 1 μ thick skin) and moreover the numerical aperture of the objective must be adequate to resolve the patterns at the two boundaries of the skin. If the skin no longer corresponds to a discrete layer but to a gradual change in properties, then, no matter what objective is used, one will not measure the index at the surface of the specimen but will obtain a value dependent upon the degree of defocusing and the gradient of refractive index.

The compensator method is not subject to these criticisms but it has a severe limitation for use with textile fibres in that the thickness of the fibre must be known. Obviously, this can only be measured with any accuracy if the fibre has a regular cross-section and is not convoluted like many textile fibres. Recently, however, Faust[68] has developed a method of overcoming this difficulty. In this method, the specimen is immersed in a liquid so chosen that its dispersion curve intersects that of the solid at some wavelength in the visible region. On either side of the specimen are two paralle semi-silvered mirrors forming an interferometer which is illuminated at normal incidence with a parallel beam of white light. An image of the immersed specimen is then projected by means of a microscope on to the slit of a spectrometer. On viewing in the normal way one can observe coloured fringes interspaced with dark bands (see Figure 10). The position of the specimen is revealed by the fact that the fringes, instead of being straight, are displaced where they cross the specimen; this displacement does, however, vanish at that wavelength at which the refractive index of the liquid equals that of the solid. In other liquids, the wavelength at which matching occurs will be different and a series of observations in a range of liquids will thus allow a dispersion curve for the fibre to be drawn. The method also provides a convenient and accurate means of finding the refractive index across a fibre and has been found most useful for showing up differences in the optical properties of the skin and the core of cellulose fibres. This technique can be used to measure the birefringence of a fibre to an accuracy of 0·0005. However, if an accuracy of this sort is to be attained the interferometer gap must be kept small and hence the technique is most useful for thin specimens.

For thick fibres, Faust[69] has devised an alternative procedure in which the fibre is placed in a wedge-shaped interferometer. In this case the fringes are formed using monochromatic light with the fibre so placed that it is at right angles to them. Having eliminated the possibility of using wavelength variations as in the previous method, Faust altered the refractive index of the liquid relative to that of the solid by changing the temperature of the system—the temperature coefficient of refractive index for a liquid generally being about ten times that for a solid. This provides very similar information to the wavelength-variation method and, as with the former technique, it is unnecessary to know the specimen thickness.

In conclusion it may be said that if it is desired to obtain reliable information about the refractive indices of the skin and the core of a cellulose fibre then the techniques employing interference microscopy described above are usually preferable to the compensator and Becke line methods.

Birefringence and Orientation

The birefringence of an ' isolated ' polymer molecule is determined by the structure of the repeating unit and the configuration of the polymer chain, and may therefore be regarded as a molecular property. In an assembly of molecules the birefringence will, in

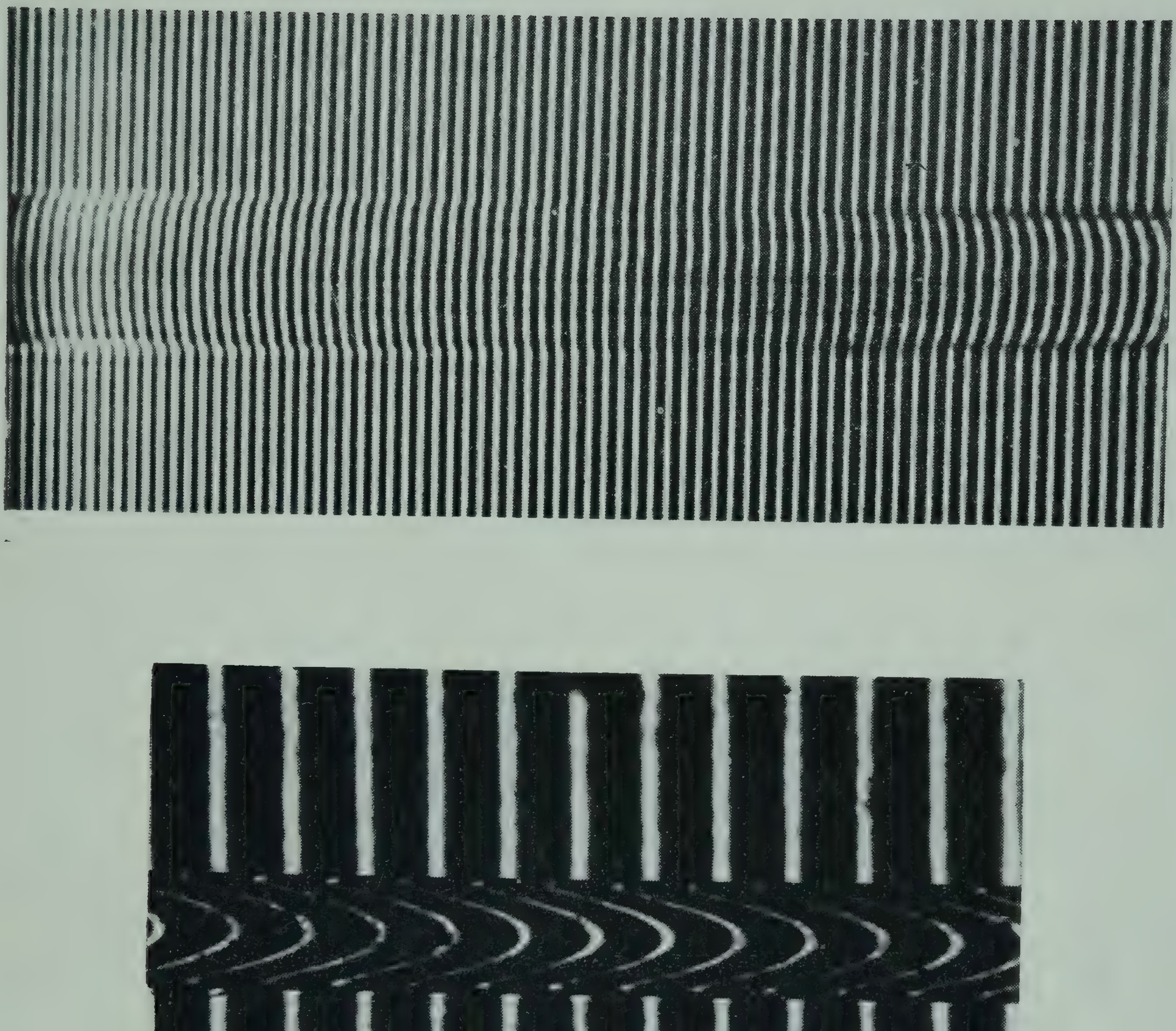


Figure 10. Determination of the refractive index of a fibre by interference microscopy. (a): Parallel plate interferometer and a nylon fibre; (b): Wedge-shaped interferometer and a viscose model filament exhibiting a skin-core structure

addition, depend on the influence that neighbouring molecules have on the polarizability of each polymer chain. This latter effect is most marked when the molecules are in a regular ordered arrangement such as is present in crystalline regions. However, even here it is usually regarded as small and to a first approximation the crystalline regions are simply regarded as regions of higher density. This assumption then allows one to calculate the birefringence of a fibre at any density by applying the well-established but empirical formula $(n_1-n_2)/\rho =$ constant, where n_1-n_2 is the birefringence and ρ the density.

The birefringence (n_1-n_2) of a fibre of density ρ in which all the polymer molecules are inclined to the fibre axis at the same angle θ is related to the birefringence $(n_\gamma-n_a)$ of a perfectly oriented specimen of the same density by the expression[70]

$$\frac{(n_1-n_2)}{(n_\gamma-n_a)} = 1-\frac{3}{2}\sin^2\theta \qquad \ldots (2)$$

(This relationship is not in fact exact as the birefringence has been used instead of the corresponding difference of polarizability, but for most practical purposes this approximation is sufficient.) In principle, therefore, a measure of an average angle at which the molecular chains are inclined to the fibre axis may be obtained from birefringence measurements. One difficulty in applying this equation is that perfect fibre orientation is not attainable in practice and the best that can be done is to use the maximum experimental birefringence that can be obtained.

The orientation of the crystalline regions of Ramie as determined[66] by the X-ray method corresponds to an orientation factor of 0·97. Hermans[71] has used this fibre to determine the birefringence of a cellulose fibre with perfect orientation, and after correcting his results to a standard density of 1·52 he found the following values.

$$n_\gamma = 1{\cdot}590^{0},\ n_a = 1{\cdot}519^{0};\ n_\gamma - n_a = 0{\cdot}071$$

Hermans also determined the birefringence of highly oriented mercerized Ramie fibres (X-ray orientation factor of 0·98) and on correcting to the standard density of 1·52 he found[71]:

$$n_\gamma = 1{\cdot}578^{2},\ n_a = 1{\cdot}523^{2};\ n_\gamma - n_a = 0{\cdot}055$$

The difference between these two values for the birefringence of a perfectly oriented cellulose fibre at a density of 1·52 raises the problem of which should be used to determine the orientation of an ordinary cellulose fibre. Hermans himself has pointed out that mercerization of the Ramie fibre may have preferentially reduced the orientation of its amorphous regions, and hence the total birefringence, and this may well have escaped detection by the X-ray method used. However, he does not think this likely for several reasons[72] and suggests that probably the real reason for the difference is that the crystalline regions of a natural fibre have a different birefringence to the crystalline regions of a mercerized fibre, due to the known existence of different crystal structures in the two cases. This does seem to be a most likely answer and, if it is so, then the birefringence of cellulose molecules in parallel alignment is not simply proportional to the gross density of the system—account must be taken of the amount and type of the crystalline material present. Thus in all work concerning the orientation of the polymer molecules in cellulose the value of the maximum birefringence used should be that obtained for a perfectly oriented fibre with the corresponding crystalline form.

Hermans has called[70] the ratio $(n_1 - n_2) : (n_\gamma - n_\alpha)$ given in equation (2) the 'optical orientation factor'. It gives a measure of the average chain orientation and it is through this relation that a comparison can be made with the orientation determined by the X-ray method.

In the above description of the birefringence, it was tacitly assumed that the textural inhomogeneities in the sample played no part. Wiener[73] has, however, shown that an assembly of small oriented particles which are optically isotropic, but of an asymmetric shape, may behave in bulk as an optically anisotropic material. He has worked out the theory for particles in the form of rods or plates immersed in an isotropic medium. This shows that, as the refractive index of the medium is changed, the birefringence of the system will vary, having a minimum value when the refractive indices of the particles and medium coincide. This minimum value measures the intrinsic birefringence of the particles—being zero if the particles are in fact optically isotropic. The measured birefringence is thus made up of the sum of this intrinsic birefringence and the 'form' birefringence due to the Wiener effect.

In semi-crystalline polymers, there exist units of structure, the crystallites, which may be supposed to be asymmetrical in shape and hence contribute a Wiener component to the birefringence. Wiener's theory does, however, assume a sharp phase-boundary between the particles and the surrounding medium, a condition which is not now thought to exist between the crystallites and the intervening amorphous regions in the polymer.

Experimentally, it would seem that in cellulose, as in most polymers, the conditions for the Wiener effect to occur are only satisfied when the samples are highly swollen.

In conclusion, one may note that the birefringence technique though very sensitive if the polymer molecules themselves possess a high intrinsic birefringence, does not provide the detailed information that can be obtained by the X-ray method. Its main limitations may be stated as follows. Firstly, it gives only an average orientation of the polymer molecules and tells us nothing about the distribution of orientations. Secondly, it does not distinguish between the orientation of the crystalline and amorphous regions. Thirdly, the formulae given here for the average angle of orientation and for the density correction are based on the assumption that the polarizability of a molecule is not affected by interaction with a neighbouring molecule, an assumption which is known to be not strictly true. Fourthly, in semi-crystalline polymers like cellulose 'form birefringence' effects may contribute to the intrinsic value. For these reasons, the results on the orientation determined by this technique should be used with care. However, the sources of uncertainty mentioned are usually second-order effects and the orientation determined by this method is likely to be of the right order of magnitude. Hence, although the values obtained cannot have absolute validity, they are most useful for comparison of one specimen with another of the same species.

Infra-red Spectroscopy

In recent years, some interesting and important results have been obtained on the orientation of the polymer molecules in fibre-forming materials by the use of polarized infra-red spectroscopy. Briefly, the method depends on the fact that certain types of vibration of a chemical group in a molecule produce an oscillating dipole moment and if this is parallel to the electric vector of the incident infra-red radiation then the absorption is a maximum. For a vibration of a chemical group which produces an oscillating dipole moment either exactly parallel or perpendicular to the polymer chain axis, the dichroism for a perfectly orientated sample ought to be complete, i.e. if the infra-red radiation is polarized firstly along the polymer chain axis and then perpendicular to this axis there should be no absorption for one of these directions. For specimens in which the orientation is less perfect, the ratio of the absorptions with the electric vector of the radiation parallel and perpendicular to some chosen axis of the specimen is a measure of the average angle that the polymer chains make with that axis. Most of the work done to date by this method has been on fibrous proteins and oriented synthetic polypeptides. Indeed it was from measurements of infra-red dichroism that a synthetic polypeptide was shown[74] to be in a folded and not an extended form. Later it was shown[75,76] that fibrous proteins could be recognized by the characteristic dichroism of the NH and CO stretching modes in their spectra.

Many polymer spectra show bands[77] which arise only from the crystalline regions. These may be recognized by observing which bands disappear when a polymer is melted or when by other means it is rendered amorphous. Unfortunately, it is often difficult to assign these bands to particular vibrations of the polymer molecule, but in the cases where it can be done and the direction of the oscillating dipole moment with respect to the polymer chain is known then the orientation of the crystalline regions may be estimated independently of the amorphous regions. Recently it has been shown[78] that in certain polymers, polyethylene and polyethylene terephthalate for example, there are also bands in the spectra which arise from the amorphous regions only, and hence the determination of their dichroic ratio allows the possibility of determining the orientation of these regions.

A somewhat different infra-red method of measuring orientation is provided by the preferential deuteration of such groups as OH or NH in the amorphous regions of a polymer. This allows the study of the orientation in the crystalline regions by examination of the dichroism of the undeuterated OH or NH groups. This method[49] has been found particularly useful for cellulose in studying the orientation of the hydrogen bonds in the crystalline regions (see p. 165). It has not yet been used to determine the molecular chain orientation for this demands a more precise knowledge of the crystal structure than we have at present. This knowledge is only necessary, however, if absolute measurements of orientation are required; if relative values are sufficient, then so long as comparisons are restricted to specimens with the same crystalline form this method offers a reliable and easy means of determining the average chain orientation in the crystalline regions.

When this work has been extended, as no doubt it will be, the infra-red method will, in favourable cases, offer the advantage over the birefringence method of being able to determine in absolute terms the average molecular chain orientation in both the crystalline and amorphous regions separately.

THE SIZE AND SHAPE OF THE CRYSTALLINE AND AMORPHOUS REGIONS

Natural cellulose fibres consist of a thin outer membrane or primary wall enclosing a thicker secondary wall, the latter constituting the major part of the fibre. It has been known for a long time that when

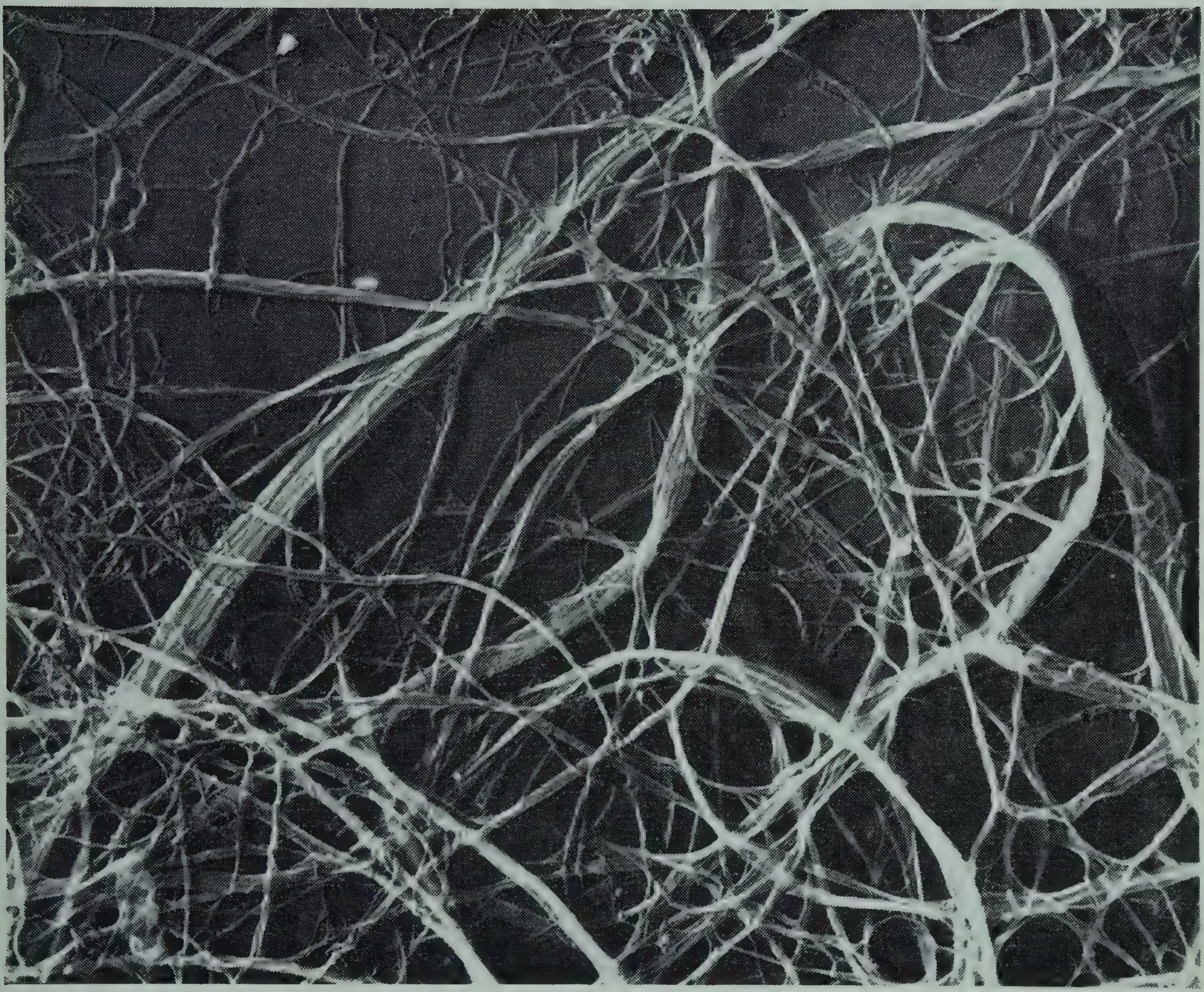


Figure 11. Electron micrograph[81] *of the fibrils exhibited by Fortisan after being homogenized for 1 hr. Magnification 15,000*

these celluloses are swollen they also exhibit a fibrillar structure which is just discernible under the optical microscope, and recent studies using the electron microscope have revealed the presence of a still finer system of fibrils.

In order to study by electron microscopy the internal structure of a cellulose sample some sort of disintegration technique is necessary. Of the methods which have been employed, mention may be made of mechanical action (beating or grinding), ultrasonic vibration and chemical degradation. Not surprisingly, there seems to be a rough correlation between the severity of the disintegration procedure and the diameter of the microfibrils observed. Usually ultrasonic treatments yield finer and more uniform fibrils than mechanical treatments, and although widely different dimensions have been reported most investigators agree that the fibrils in natural celluloses are extremely long, sometimes up to several thousand Ångströms, with an average diameter of around 80–150 Å.

Although most work has been done on natural celluloses, microfibrils are also observed when mercerized fibres are examined in a similar way. Ordinary regenerated fibres do not however separate into fibrils[79] unless they have been highly oriented during manufacture.[80] The fibrillar structure exhibited by Fortisan is shown in Figure 11.

When a cellulose which exhibits fibrillation is degraded chemically the electron micrograph reveals the presence of small elongated tabular particles[80,82–85] with a width of the order of 50–100 Å, a thickness of about 20–50 Å and a length which may vary from about 100 Å to several thousand Ångströms. The length of these particles is reduced by more drastic or prolonged acid treatment but their lateral dimensions remain practically unaffected. A typical electron micrograph of the particles obtained by the acid degradation of cotton fibres is shown as Figure 7 of the previous chapter.

It is thought that these particles are fragments of the fibrils which have survived attack by the acid and consequently represent the more ordered regions. That these particles are in fact crystalline has been confirmed by X-ray diffraction, and in addition it is interesting to note that the breadth of the equatorial X-ray reflections of various types of untreated cellulose fibres decreases in the order that one would expect from the lateral dimensions of the particles.[11] Thus although it is thought that the particles are closely related to the crystallites present in the untreated fibre conclusive evidence is lacking because it is uncertain what effect the chemical or mechanical treatment has on the original structure.

A more direct method of obtaining information on the original structure is provided by examination of the breadth of the X-ray reflections. It is well known that the reflections given by the crystalline regions of high polymers are less sharp than those given by large perfect crystals. Diffraction theory shows that when the crystals are less than about 1,000 Å across, the broadening becomes appreciable and increases with decreasing crystallite size according to the expression[86]

$$\beta = \frac{0{\cdot}9\,\lambda}{t}\sec\theta$$

where β is the breadth of the reflection at half the maximum diffraction intensity, λ the wavelength, t the size of the crystallite, and θ the Bragg angle of diffraction.

Unfortunately, the size of the diffracting crystal is not the only possible cause of the broadening of an X-ray reflection, other effects such as the thermal motion of the molecules or imperfections in the crystal will contribute. It is not, however, always possible to distinguish between these various factors but nevertheless it is probably justifiable to regard the crystal size as calculated in this way as the approximate size of the undistorted crystalline regions.

Experimentally, it is found that the reflections for wood are very broad, for cotton they are of medium width and in Valonia cellulose they are very sharp implying that the average crystal size increases in the order given. Different crystal reflections for one cellulose may show different degrees of broadening indicating that the dimensions of the crystals are not the same in all directions. Estimates[87] that have been made of the crystallite size in Ramie indicate that they are long and rod-shaped with a diameter of about 50–100 Å and a length of at least 500–600 Å. Due to the uncertainties of this method, however, these figures, although in reasonable agreement with data from the electron micrographs, should only be regarded as approximate.

An alternative X-ray method[88,89] which is used for gaining some information on this aspect of structure is the study of the distribution of X-rays diffracted at very small angles. It is thought that the low-angle diffraction depends simply on differences of average electron density in the specimen and is independent of crystal imperfections and thermal motions of the atoms and, consequently, is more directly related to gross structure. If the scattering shows a maximum this means that there is some regularity or periodicity in the spacing of the crystalline and amorphous regions. In this case, it is customary to employ the Bragg equation to determine this spacing. On the other hand if the scattering shows no maximum then for solid specimens such as fibres the interpretation is difficult and has been the subject of much controversy.[90,91]

Although the theory of small-angle scattering is not entirely satisfactory the results obtained by this method indicate a particle size of 50–100 Å showing reasonable agreement with other techniques.

Recently, Hermans and Weidinger[89] have examined the small-angle X-ray scattering of swollen rayons. It was found that in all cases where the water-swollen fibre shows a maximum in the scattering this maximum shifted to lower angles on increasing the swelling by immersion in lithium hydroxide solutions. This is what would be expected if the maximum is due to an often recurring interparticle distance which is increased upon further swelling. A plot of the scattering observed for Fortisan is shown in Figure 12.

With 5N lithium hydroxide, intracrystalline swelling takes place, as shown by the wide-angle X-ray scattering, thus accounting for only an inflection in the curve. Hermans and Weidinger do point out however that in some cases the maximum disappears at a much lower concentration and thus the above explanation cannot be a

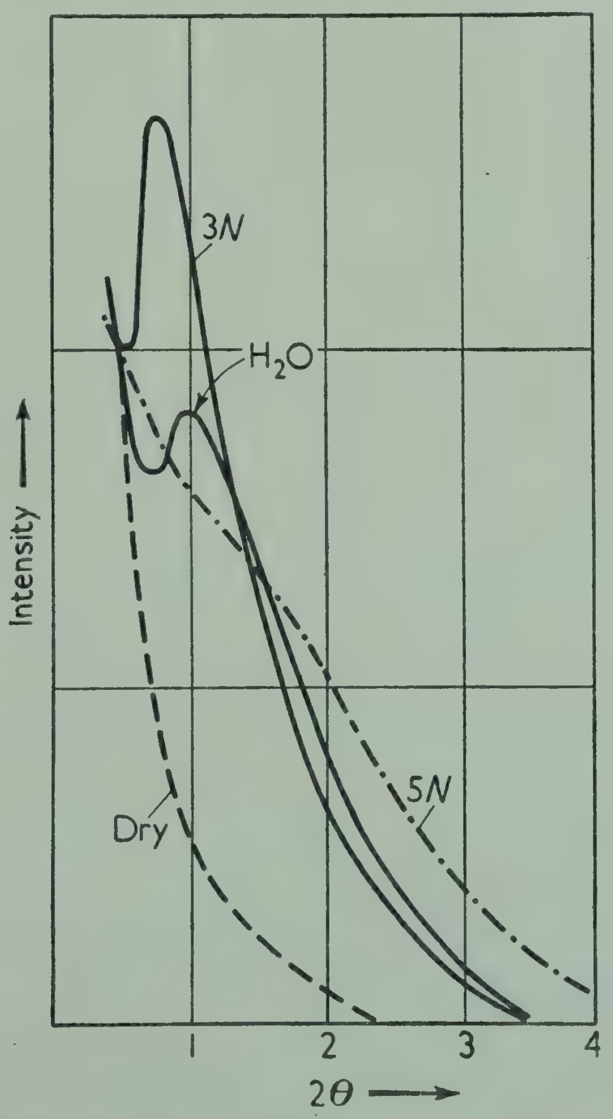


Figure 12. Small-angle X-ray scattering[89] *of Fortisan swollen in lithium hydroxide solutions*

general one. Some of the results for various types of rayons that these authors have examined are shown in Table 7.

Using a model, proposed by Kratky and Porod[90] of lamella particles approximately parallel to one another, Hermans and Weidinger[92] find that their results lead to a lamellar thickness o between 25 and 40 Å if a swelling ratio of 2·0 in water is assumed.

Table 7: Small-angle scattering of swollen rayons[89]

Material	Swelling medium	Type of curve	Location of maximum	
			2θ	Bragg period Å
Fortisan... ...	Water	Maximum	1·04	84
	2N LiOH	,,	0·81	110
	3N LiOH	,,	0·84	100
	5N LiOH	Inflection point	—	—
Fortisan H ...	Water	Maximum	0·91	97
	3N LiOH	,,	0·71	124
	5N LiOH	Inflection point	—	—
Fiber G... ...	Water	Maximum	1·2	74
	3N LiOH	,,	0·74	118
	5N LiOH	Inflection point	—	—
Celanese X36 ...	Water	Maximum	1·13	78
	3N LiOH	,,	0·78	114
	5N LiOH	Inflection point	—	—

These authors[92] have also examined the small-angle scattering of Fortisan after various periods of mild heterogeneous acid hydrolysis. They find that the position of the maximum in the scattering after 400 hr of hydrolysis remains unchanged but becomes very sharp and pronounced, and state that no special model or theory is required to justify the conclusion that the fibre has a periodic structure with a periodicity that can be derived to a very close approximation from the diffracting angle of the peak according to Bragg's law. This leads to an interparticle distance of 80 ± 4 Å in water-swollen Fortisan. There seems to be little doubt that the periodicities measured are intimately related to the occurrence in the fibres of crystalline and non-crystalline regions of a remarkably regular size.

Less direct evidence on the size of the crystalline regions in cellulose can be obtained from the levelling-off degree of polymerization during hydrolysis.[3,80] The values obtained by Morehead[80] for the average length using this method agree remarkably well with the average values determined by direct electron microscope observation.

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7

CELLULOSE DERIVATIVES

INTRODUCTION

The possibility for really novel advance in the chemistry of cellulos derivatives is greatly restricted by the sensitive and vulnerabl nature of cellulose itself, and it is not easy in examining the wor of the past few years to uncover very much that could not b described as mere variation on the old theme of esterification an etherification. Any treatment which results in the breaking of th C—C or C—O bonds of the pyranose rings will not give a tru derivative of cellulose, and reaction is therefore very much restricte to attack at the three hydroxyl groups of the anhydroglucose unit

Again, a good deal of work has recently been carried out with view to modifying cellulose in the form of cotton goods. Variou after-treatments have been evolved designed to confer on cotto certain properties such as ease of drying, crease-resistance, or non inflammability. Although there is evidence that in many cases th resins or other intermediates used do attach themselves chemicall through the hydroxyl groups of the cellulose, extent of reaction i often small, and the product is more properly described as a modifie cotton or cellulose, rather than as a cellulose derivative of well defined character. It is intended, therefore, to exclude most of suc superficial, though very important, treatments, and to deal wit those in which extent of reaction results in large measure in a entirely new chemical product.

The esters and ethers which form the subject of much of this sectio are in many cases not new, but the past ten years have provided period in which relative importance has been thoroughly tested an in which advance in commercial utility and practice can here an there be recorded.

CELLULOSE ESTERS

Cellulose Acetates

At least from a commercial point of view, one of the most impo tant advances in recent years in the field of cellulose derivatives h been the tardy recognition of some of the virtues of cellulose triacetate. Through improvement in industrial techniques and better understanding of the fundamental structures and chemistry of the cellulose acetates, this sixty-year-old material has now attained a growing commercial importance, and newspaper and magazine advertising will have made familiar the name of Tricel, the only cellulose triacetate fibre on the British market at the present time. An equivalent material has been developed in the United States under the name of Arnel.

The character of cellulose is such that esterification, if carried to completion, will produce a triester by reaction at each of the three hydroxyl groups of the anhydroglucose unit. This, indeed, is what the early investigators achieved, although in a rough and ready way, since at that time control of esterification was not understood as well as it is today.

Cellulose triacetate was tried as a commercial proposition as long as 50 years ago, when in Boston, Massachusetts, U.S.A., a small plant began to turn out about a ton of continuous filament yarn per week. The venture did not last long, partly due to the hazards of operating with chloroform as major solvent, and partly to lack of experience in the dyeing of a relatively hydrophobic fibre. Also, to those with experience of the difficulties which can be caused by incomplete removal of catalyst from a cellulose derivative, it seems possible that the early triacetate had poor stability to heat and to hydrolysing conditions, due to the presence of sulphate groups in the yarn, sulphuric acid being the catalyst used in the esterification reaction.

These difficulties, and the resulting commercial failure of cellulose triacetate, were soon forgotten in the enthusiasm aroused by the discovery of Cross, Bevan, and Miles that controlled hydrolysis of the primary triacetate led to a so-called secondary acetate of less hydrophobic character and with an attractive solubility in the non-toxic solvent, acetone. Whereas the triacetate has an acetic acid yield of approximately 62 per cent (i.e. the weight in grams of acetic acid produced by the hydrolysis of 100 g of the ester), the secondary acetate yields roughly 54 per cent, representing the esterification of about 2·4 hydroxyl groups per anhydroglucose unit.

There followed, then, a period of 40 years in which triacetate was in general neglected. Towards the end of this time, however, several factors began to combine to re-awaken interest. Firstly, availability of methylene chloride, and its large-scale use in the acetylation of cellulose, made tolerable by improved plant techniques, suggested that its use as a solvent for spinning triacetate need no longer pose earlier problems. Secondly, the use of methylen chloride as an acetylation solvent permitted the use of relativel small amounts of sulphuric acid as catalyst, and provided acetylatio conditions in which preparation of a fully-acetylated cellulose wa much easier. Thirdly, the rather higher crystallinity of triacetat as compared with secondary acetate, could now be recognized, a in the case of nylon and Terylene, to be of potential importance.

Three principal methods exist for the preparation of triacetat on a commercial scale. Two of these involve solution of the cellulos in the acetylating mixture, and one is heterogeneous in that th cellulose retains much of its original form and does not dissolve.

First of all, there is a modification of the process developed in thi country by the Dreyfus brothers[1] and designed originally to furnish acetone-soluble secondary acetate. Since secondary acetate wa required it did not matter in the least that relatively large amounts o sulphuric acid were used as catalyst—say, some 14 per cent on th weight of the cellulose—since, during the hydrolysis to secondary acetate, there was plenty of time and opportunity to remove such sulphate as might be combined with the cellulose.[2] When, however, the need for the triacetate arose, combined sulphuric acid at the end of the acetylation became something of an embarrassment.

In the Dreyfus process, the ester which exists in solution in the acetic acid–acetic anhydride–sulphuric acid mixture at the moment of complete esterification is a mixed sulphate–acetate. By destroying the anhydride with water and making the mixture slightly aqueous, the combined sulphuric acid is relatively easily removed but is, unfortunately, replaced by hydroxyl groups. Simultaneously, some acetyl groups are similarly displaced and the best that can normally be obtained is an acetate with an acetic acid yield considerably below the theoretical for triacetate, for example, 59 per cent or lower.

However, by graduated additions of magnesium acetate and of water,[3] and by use of temperatures in the range of 50°–100°, removal of sulphate groups and their partial replacement by acetyl can be accomplished, and commercial triacetates of about 61·5 per cent acetic yield achieved. The magnesium salt removes the sulphuric acid as the relatively insoluble magnesium sulphate.

The second process for triacetate is the one developed largely in Germany just before 1939, in which the solvent for the cellulose ester formed is methylene chloride.[4] This is so superior to glacial acetic acid as a solvent for cellulose triacetate that it permits the use of very small quantities of sulphuric acid as catalyst, as low as 1 per cent on the weight of cellulose used. The product formed in solution is much more nearly a true triacetate, combined sulphate being small. There is no need to resort to magnesium sulphate precipitation, triacetates of approximately 62 per cent acetic yield being obtainable simply by a process of very mild hydrolysis.

This methylene chloride process of acetylation lends itself also to the use of catalysts, such as perchloric acid, which are not so readily applied to those acetylations where acetic acid is the principal solvent medium. Perchloric acid, which is now used commercially, gives triacetates of almost theoretical acetyl values and which are free from any instability due to presence of mineral acid residues. Perchloric acid does not itself combine with cellulose.

A third process for making cellulose triacetate is that which was used by Schering A.G. in Germany. Acetylation is carried out as usual with acetic anhydride, but solution of the reacting cellulose is prevented by using a non-solvent medium, such as benzene.[5] The mixture of benzene and acetic anhydride should be such as to swell the cellulose to an optimum extent, so that the process of complete esterification is not unduly prolonged. The normal catalyst in this process is perchloric acid, and triacetates of theoretical acetyl value are obtained. The product has very much the appearance of the original cellulose. Incidentally, although the methods which cause the cellulose to pass into solution do not entirely preclude the attainment of esters of high molecular weight, it is probably true to say that such products, if desired, are more readily obtained by the Schering process.

A process of producing a yarn having many of the properties of cellulose triacetate is that which is being developed in Japan. This is the acetylation of cellulose in suitable textile form, say, as viscose yarn or staple, with acetic anhydride vapour. The cellulose material is impregnated with a mild but effective catalyst such as potassium acetate and, although the product is not uniform, at least the outer part of the fibre and the more accessible regions are completely acetylated and the textile product has the desired properties of triacetate. The heterogeneity is shown by the acetic yield of around 45 per cent. Acetylation is thus far from complete.

It is perhaps relevant to mention the process of partial acetylation of cotton goods.[6] The intention in this case is not to convert to a triacetate, but to confer, by acetylation of the chemically-accessible regions of the fibre, a resistance to heat and to rotting by micro-organisms not possessed by the original cellulose. Much more work has recently been carried out on this subject by the Southern Regional Research Laboratory, New Orleans, Louisiana, U.S.A. The cotton goods are treated by passing through an acetylation bath, containing acetic anhydride and a non-solvent, so tha superficial acetylation takes place.

Processes for the conversion of cellulose triacetate into usef textile products will be given only brief description here. The bul of triacetate yarn is made by dry-spinning from a solution in methylene chloride–alcohol solvent. ' Dry-spinning ' is the term use to describe processes in which a solid polymeric substance, such a cellulose triacetate, is dissolved in a solvent and the resulting solutio forced through the holes in a spinning jet into an atmosphere a such temperature that the solvent is evaporated and the polymer i formed into filaments. However, with the demand for triacetate the character of the acetylation reaction solution or ' dope ' is no such that wet-spinning of triacetate yarn direct is becoming commercial possibility. This obviates the need for precipitating washing, drying, and re-dissolving the triacetate flake and avoids the use of the relatively expensive and volatile chlorinated solvent. The acetylation ' dope ' in the process in which acetic acid is the solvent, is extruded into dilute acetic acid through suitable spinning jets, and the coagulated filaments are washed and processed in conventional manner. If adequate control can be achieved over processes of continuous acetylation, the continuity would be complete from the cellulose right through to the finished triacetate yarn.

A considerable amount of work has been done on the melt-spinning of cellulose triacetate.[7] The latter has a much sharper melting behaviour than the secondary acetate, and it can be successfully extruded at temperatures around 290°–320°, provided that the time spent in the molten state is kept short. This is absolutely essential since, although the quality of cellulose triacetate is today very high, the polymer is inherently unstable to heat. Melt-spun filaments, reproducing the shape of the holes through which they were spun and subjected as they are to a very considerable ' draw-down ', have circular cross-section, and this gives the yarn physical properties rather different from those of dry-spun yarn. ' Draw-down ' is produced by winding the filaments on to a bobbin, or other suitable holder, at a linear speed in excess of the linear rate of extrusion from the jet-hole. But many difficulties remain to be overcome before cellulose triacetate can be melt-spun to give commercially acceptable yarns.

From X-ray diffraction photographs of secondary cellulose acetate yarn it is known that the dry-spinning process used produces little orientation of the macromolecules in the direction of the filament axis, and there is also very little crystallinity. Equally there is little orientation displayed by dry-spun triacetate filaments.

but they are more crystalline (Figures 1 and 2). This is not surprising, since the possibility for order among fully esterified cellulose molecules is greater than in secondary acetate, where the hydroxyl groups may be haphazardly arranged with respect to the acetyl groups.

It was shown as long ago as 1942 that significant changes could be effected in the crystalline structure of cellulose acetates by heat treatments,[8] and, in 1948, workers of the Celanese Corporation of America reported such changes with triacetate. Later work by British Celanese Ltd and others in this country showed that these changes in crystalline structures resulted in the acquisition of valuable new properties by triacetate fabrics.

The first of these was of great importance. By heat treating cellulose triacetate textile materials for short periods of time at temperatures from 180°–280° a more regular structure is induced (Figure 3) and the softening point and resistance to glazing are raised. The safe ironing temperature goes up from 180° to about 240° after treatment.

Another new property is that heat treatment, by inducing molecular mobility, eliminates any stresses within the triacetate and permits rearrangement of the molecules in any desired way. That is to say, the arrangement of the individual fibres in a yarn or fabric can be ‘ set ’ by heat treatment so as to become the natural and strainless configuration. Thus permanent pleating of fabrics of triacetate can be achieved.

It is not contended here that imposition of permanent pleating goes hand in hand with a great increase in crystallinity. Quite satisfactory permanent pleating can be achieved without significant increase in crystallinity although there is always some indication of higher order. In degree of order and crystallinity shown, X-ray photographs of triacetate and secondary acetate resemble those of wool, and this similarity extends to certain of the textile properties (Table 1). The tensile characteristics are seen to be alike in stress–

Table 1: Tensile characteristics of wool, secondary acetate, and triacetate

Fibre	65 per cent R.H.	
	Tenacity g/denier	Extension (per cent)
Wool	1·1–1·4	40–28
Secondary acetate	1·2–1·4	30–20
Triacetate	1·2–1·4	25–20

strain diagrams for these fibres (Figure 4), and when the fibres are tested in the wet state they all show similar falls in tenacity and increased extensibility.

Before leaving this brief reference to X-ray diffraction studies of triacetate, it is interesting to look at a photograph of highly crystalline and oriented triacetate. This is of a laboratory specimen produced by heat-treatment and drawing, and does not at all represent the commercial yarns, but it does provide an example of fibre orientation and crystallinity which has probably never been surpassed (Figure 5).

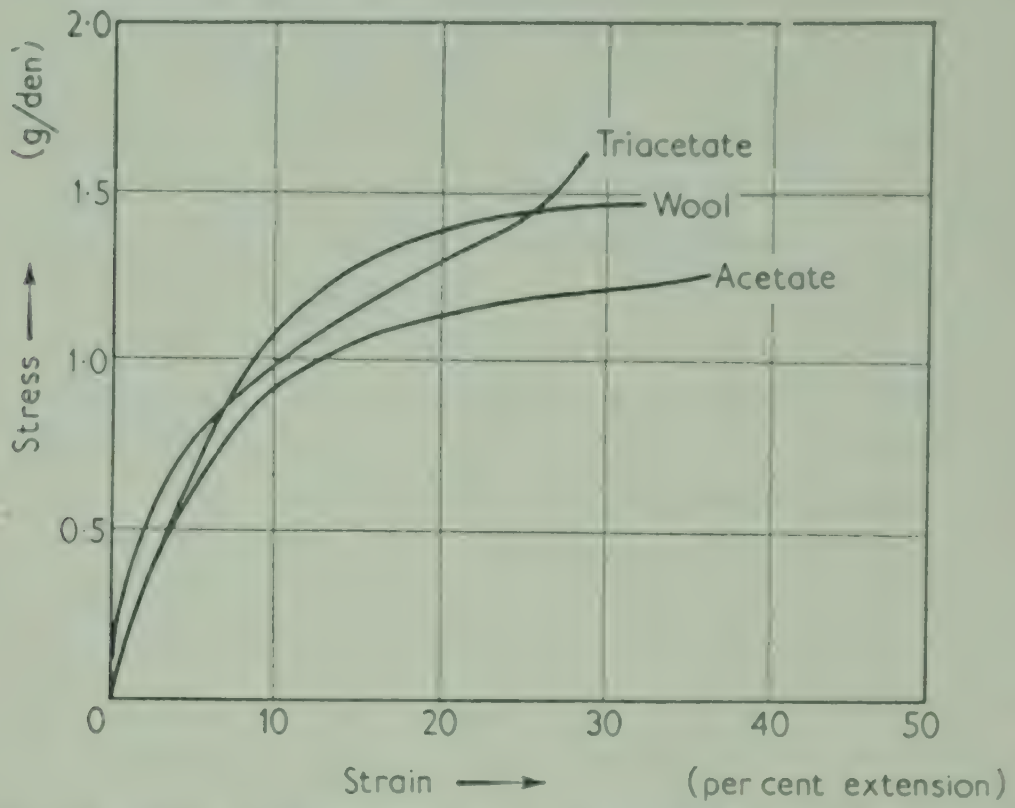

Figure 4. Stress–strain curves of acetate (secondary), triacetate, and wool

Low-acetyl Acetates

Before proceeding to other triesters of cellulose, mention should be made of recent interest in what are called ‘ far-hydrolysed ’ cellulose acetates.[9] These are mostly prepared from conventional acetylation reaction solutions, but by careful control of hydrolysis conditions, involving steady increase in the amount of water in the system, water-soluble acetates with 18–26·5 per cent acetic acid yield may be produced. With 33·5 per cent combined acetic acid, the material is insoluble in water and also in acetone. Thus, a whole range of low-acetyl cellulose acetates can be prepared having solubility in acetone or in water or in mixtures of these two solvents.

These materials are not at present made on a large scale, but they have many potential outlets, such as for coatings, either water-

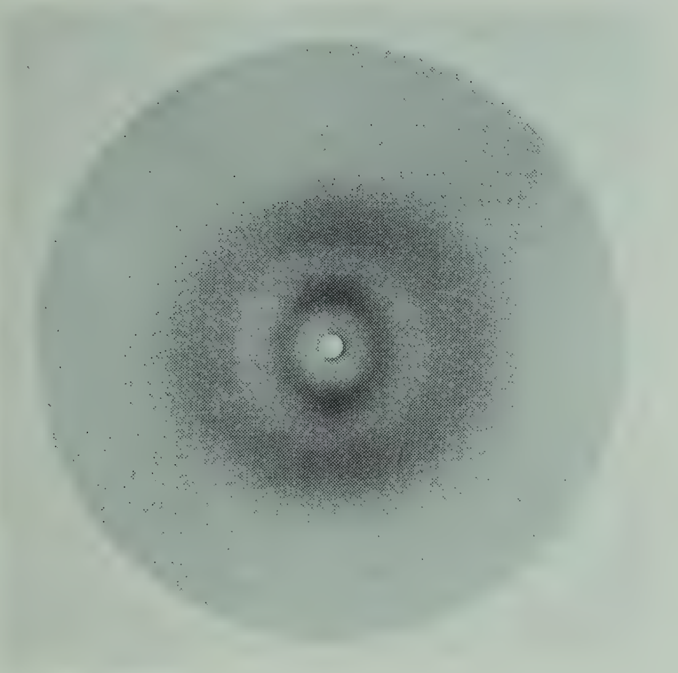

Figure 1. X-ray diffraction photograph of secondary cellulose acetate filaments

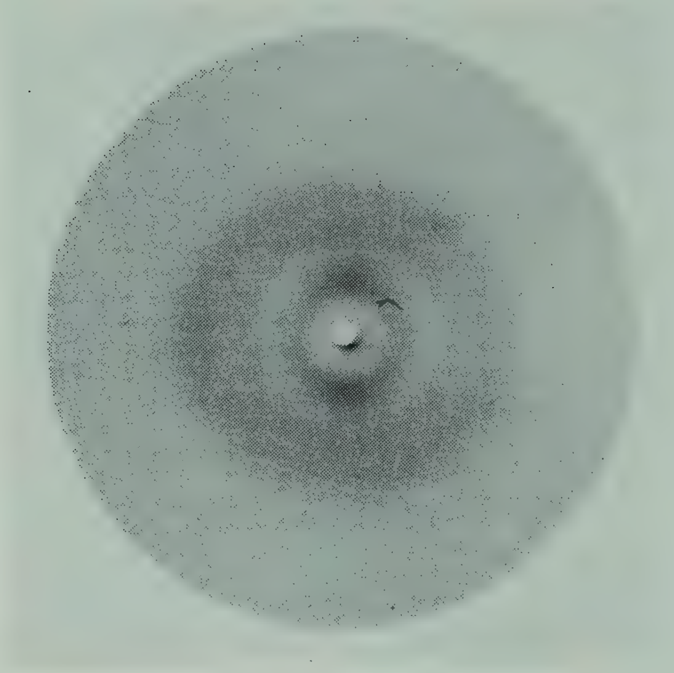

Figure 2. X-ray diffraction photograph of cellulose triacetate filaments

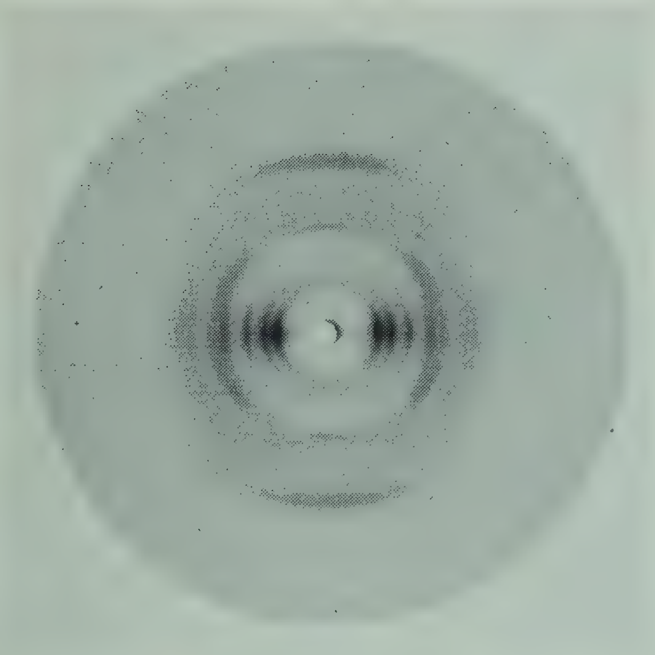

Figure 3. X-ray diffraction photograph of cellulose triacetate filaments after heat treatment

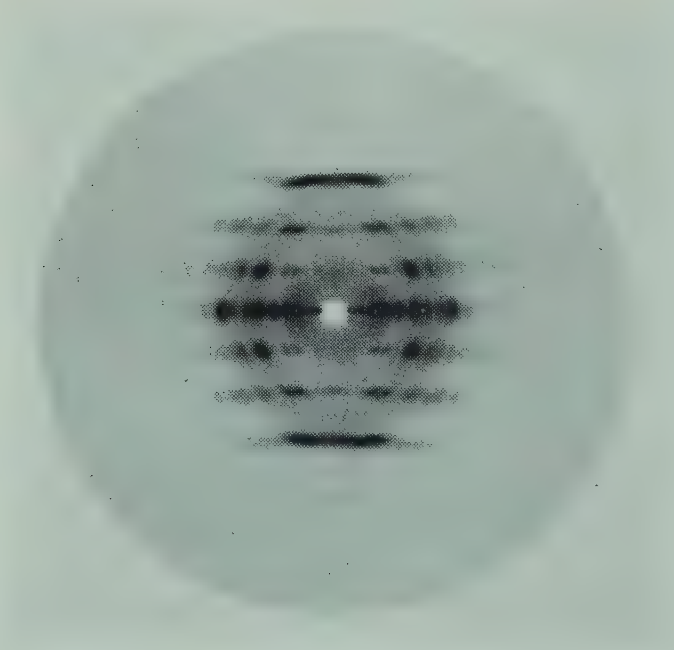

Figure 5. X-ray diffraction photograph of highly oriented and crystalline triacetate filaments

soluble or water-insoluble, in photographic applications and as thickeners and sizes.

Cellulose Sulphate

Enough has already been said of the role of sulphuric acid in cellulose esterification to justify mention here of cellulose sodium sulphate, which has been produced commercially in the U.S.A.[10] With a rather low chain length and a degree of substitution of 0·3–0·4, the sodium salt is soluble in hot and cold water. It is not a very stable material, however, and it suffers from a tendency to undergo further slow degradation.

Cellulose Propionate

This material, which has been extensively investigated in America, has not become of great commercial importance, perhaps because esters containing both acetate and propionate, or acetate and butyrate, groups can be found to do all or most that the propionate can do. The mixed esters are also rather more easily prepared for the following reasons.

The esterification of cellulose with propionic acid and anhydride, especially where sulphuric acid is the catalyst, is a slower reaction than acetylation. In such a reaction, the combination of sulphuric acid with the cellulose, an important first step, does not take place with the same ease as in acetylation conditions, so that solution of the cellulose by esterification is retarded. Quick and smooth combination of the sulphuric acid with the cellulose is important and, without this, degradation of the cellulose is caused by the unreacted mineral acid catalyst. Moreover, in the pre-treatment stage essential in the esterification of cellulose, propionic acid is inferior to acetic acid as a swelling agent, and this again operates against quick esterification and provides time for degradation of the cellulose. Thus it is that most cellulose propionates have in reality been acetate–propionates, as it has been customary to swell the cellulose with acetic acid with the inevitable result that some acetyl groups enter into combination. Moreover, completion of the esterification is often assisted by a deliberate addition of acetic acid near the end of the process and this accounts for the presence of some of the combined acetic acid in the cellulose ester.

Although generally containing some unesterified hydroxyl, commercial cellulose propionate is usually near to the triester, but shows considerably greater solubility than an acetate of corresponding degree of esterification. The step from acetate to propionate gives the first indication of the greater solubility range that can be achieved by the use of the higher aliphatic acids. The substitution of propionic for acetic acid results in the acquisition of solubility in cyclic ethers, ketones, esters, organic nitrogen compounds, and even in benzene, and when the combined organic acid has from six to eight carbon atoms, solubility in alcohols is achieved.

Cellulose tripropionate, or near triester, in either flake or filament form, is distinctly softer than triacetate. It has a very much lower melting point, about 230°; and a lower softening point than commercial secondary acetate. This, together with the lower tensile strength of yarns made from it, has so far discouraged its use for textile purposes. The slightly hydrolysed ester, with about 0·2–0·3 hydroxyl group per glucose unit, with a melting point of about 210°–220°, has found some commercial outlet in America, where its greater dimensional stability has made it superior to acetate for certain plastics uses.[11]

Cellulose Butyrate

This has never attained commercial importance, probably because acetate–butyrates are more easily prepared and go as far as is necessary in providing the desired properties.

Esterification of cellulose with butyric acid and anhydride is even more difficult than esterification to give propionates, and this is particularly so when sulphuric acid is used as catalyst. Not only is butyric acid a poor swelling agent for cellulose but, in its presence, combination of the sulphuric acid with the cellulose is so greatly impeded that esterification becomes difficult. If water is used with the butyric acid during the pre-treatment stage, at least better swelling is secured, but, since water destroys by hydrolysis some nine times its own weight of butyric anhydride, there have always been objections to this procedure on the grounds of economy.

There are other catalysts which, for the preparation of butyrates, are at least as effective as sulphuric acid when used with the proper esterification conditions. But with the exception of perchloric acid, which modern industry now regards as relatively safe, there are objections to all of them.

Cellulose tributyrate, in moulded form, is a very soft material compared with cellulose acetate. It has a greatly enhanced solubility in solvents and compatibility with plasticizers, but suffers from a low melting point of about 185°.

Cellulose Mixed Esters

As will already have been inferred, the commercially important mixed esters of cellulose are those containing a combination of acetic acid with either propionic or butyric acid. They have not been produced commercially in the U.K., but have had considerable application in the U.S.A. where the Hercules Powder Company and the Eastman Kodak Company (Tennessee Eastman) have provided them in the Hercose and Tenite series respectively. In Germany, too, Farbenfabriken Bayer A.G. have for some time provided Cellit B, an acetate–butyrate, in market quantities. Acetate–butyrate, as a typical mixed ester, will now be dealt with in greater detail.

Cellulose Acetate–Butyrate

This is generally manufactured by esterifying cellulose with a mixture containing both acetyl and butyryl radicals, with the usual requirement that there shall at least be sufficient anhydride present to react with the water eliminated during esterification. The composition of the final cellulose ester with respect to combined acetic and butyric acids depends very largely on the composition of the acylating mixture, which must be adjusted in accordance with established proportions. For any desired proportion of acetyl to butyryl in the ester to be made, there is a required composition in the esterifying bath. However, it does not matter whether the necessary anhydride is introduced as butyric or acetic, so long as the overall principle of correct bath composition is not violated.

Sulphuric acid is generally the most useful catalyst, but as mentioned before, it does not combine well with the cellulose in the presence of butyric acid.[12] It is therefore desirable to conduct pre-treatment of the cellulose in the presence of acetic acid only, to ensure maximum swelling, and then to effect the esterification by using more anhydride and more sulphuric acid than is necessary in a simple acetylation. If this be coupled with a restriction of the overall quantity of reagents to the minimum possible, esterification can be carried through reasonably quickly to give an acetate–butyrate of desired composition and of acceptable viscosity.[13]

It will be seen that the preparation of acetate–butyrate demands more complicated consideration than does that of acetate. Perhaps some simplification is afforded by the German practice of working with the mixed anhydride, acetic–butyric anhydride, made by passing ketene into butyric acid. Instead of supplying all the anhydride in the form of the cheaper acetic anhydride and then having to offset this by incorporating large quantities of butyric acid, the necessary anhydride may be supplied as acetic–butyric, thereby reducing the further quantity of butyric acid needed to give an acylating bath of the desired composition.

The relationship of bath composition to ester composition and the combination of sulphuric acid with cotton cellulose in the presence of acetic and butyric acid are illustrated in Figures 6 and 7.

Cellulose acetate–butyrates are available commercially with a range of butyric acid contents but the most common are those having about 17 per cent, 27 per cent and 37 per cent, respectively. In all cases, the esters are partially hydrolysed so as to contain from

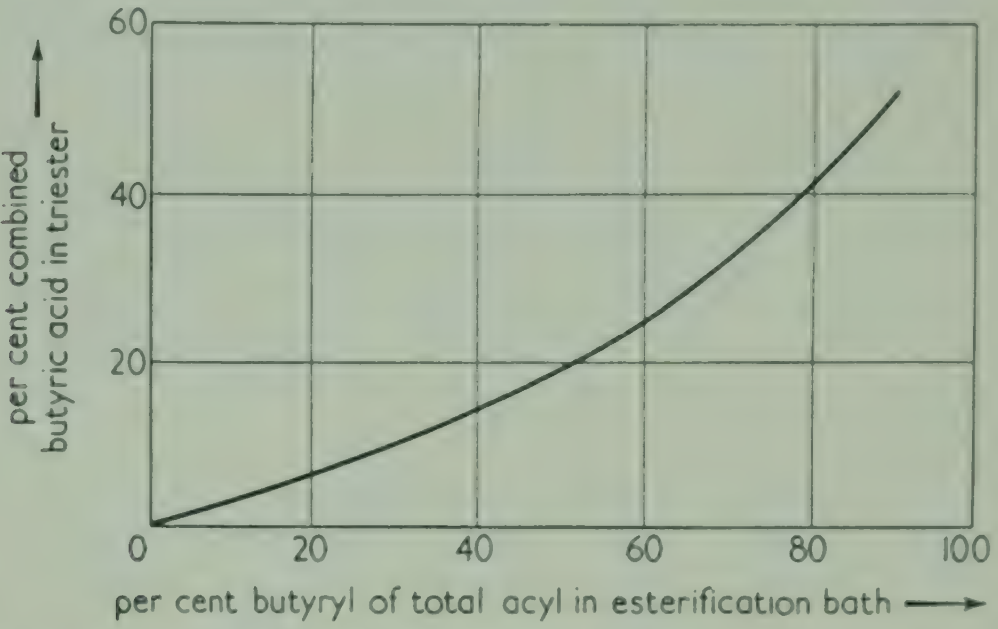

Figure 6. Composition of esterification mixture vs per cent butyric acid in ester

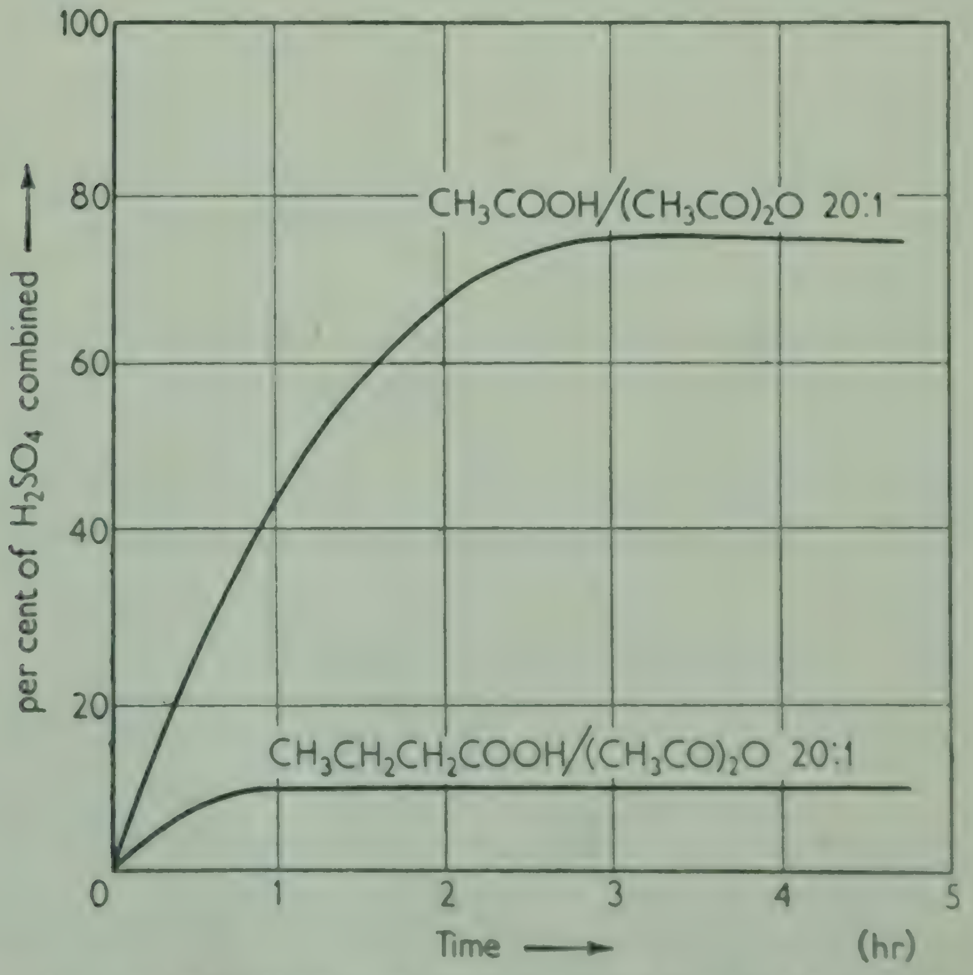

Figure 7. Combination of sulphuric acid with cotton (1 part cellulose to 20 parts liqu containing 0·5 per cent H_2SO_4)

0·3–0·5 hydroxyl group per unit. In the case of the German Cellit B, for example, the composition was 43 per cent combined acetic and 17 per cent butyric acid, with about 0·5 unesterified hydroxyl group per unit. The higher the butyryl content, the greater the range of solvents and compatibility with plasticizers. This latter is important since the acetate–butyrates are used exclusively for plastics applications. For equivalent degree of esterification, the higher the butyryl content the greater is the moisture resistance and consequent dimensional stability. It will be seen that a nice balance in composition is required to secure for any particular end-use the desired solubility, compatibility, hardness, strength, and dimensional stability.

It is this matter of dimensional stability which led some users of the cellulosic compounds away from cellulose acetate, which owed its original manufacture in this country to a demand for a relatively non-inflammable coating for aeroplane wings. However well it may have replaced the inflammable cellulose nitrate, it was certainly much inferior to the latter in dimensional stability, so it is not surprising that for this and other similar uses the search went on for a product which could combine something of the dimensional rigidity of cellulose nitrate with the relative non-inflammability of acetate. Stability of dimension to varying conditions of humidity is essential in many plastics applications, and especially in films and coatings, and is closely connected with the moisture uptake or ‘ regain ’ of the material. Although moisture regain is affected by the presence of plasticizers, it is interesting to see how the various cellulose esters themselves fit into a scale in which commercial cellulose acetate of 53·5 per cent acetic acid yield is given as 100 (Table 2).

Table 2: Moisture regain values for some cellulose esters

Ester	Regain
Cellulose acetate (53·5 per cent A.V.)	100
Cellulose triacetate	66
Cellulose acetate–propionate (Hercose AP)	53
Cellulose acetate–butyrate (Tenite II)	43
Nitrocellulose (11·8 per cent N)	23
Cellulose acetate–stearate (27 per cent stearic acid)	18

The figures are approximate only, but they do show the relative affinities for moisture.

The effect of unesterified hydroxyl groups on the melting point of acetate–butyrate depends on the relative amounts of combined acetic and butyric acid. Cellulose acetates fall rapidly in melting point until about 1 hydroxyl group per glucose unit is present, whereas cellulose butyrates have a minimum melting point with about 0·4 hydroxyl group per unit, after which, with increasing hydroxyl content, the melting point rises rapidly.

Cellulose Acetate–Stearate

These mixed esters are not made commercially, but have been reported in the literature.[14] Without special techniques, such as by the use of acid chloride, or by the ' impelling ' action of chloracetic anhydride or trifluoracetic anhydride, it is not easy to get stearic acid into combination with cellulose. These are examples of certain substituted anhydrides which, while they do not themselves esterify cellulose, can be used as impelling agents to bring about reaction between cellulose and unreactive acids.[15,16] However, the particular acetate–stearates under discussion were not required to have a large stearic substitution per anhydroglucose unit, so that it became feasible to produce them by esterification of the free hydroxyl groups in commercial cellulose acetate. The procedure was to dissolve an appropriate secondary acetate in methylene dichloride and then to add the required quantity of stearic anhydride with perchloric acid or zinc chloride–hydrochloric acid as catalyst. The stearic anhydride was produced by reacting stearic acid with acetic anhydride, sometimes in a toluene medium, and distilling off the lower boiling acetic acid or its toluene azeotrope. With these conditions a smooth esterification of the hydroxyl groups was effected, and acetate–stearates with up to 40 per cent combined stearic acid were readily made. The ester principally investigated was one in which the stearic acid content was about 27 per cent, and which had about 2·3 acetyl groups, 0·35 stearyl and 0·35 hydroxyl groups per glucose unit.

This mixed ester was soluble in a wide range of solvents, had a melting point around 200°, and gave from suitable formulations soft flexible films of very high dimensional stability. As will be seen from Table 2 it had a very low moisture regain. It was compatible with all the conventional plasticizers used for this type of material, and gave particularly attractive coatings and films when plasticized with a vegetable oil, such as castor oil, as a major constituent.

Other Mixed Esters

It is not possible to deal within the scope of this paper with all the various esters and mixed esters of cellulose which have been made and examined in one laboratory or another. The literature on the subject abounds with references to caprates, laurates, and palmitates, to benzoates, succinates, and sorbates, and to cellulose mixed esters containing these acids and acetic acid.[17] It is proposed, therefore, to restrict mention to two more of which the author has had some experience. The first of these is that in which cellulose is esterified partly with acetic acid and partly with a dibasic acid, in this case phthalic acid. Again for the purpose required it was sufficient and convenient to begin with a secondary cellulose acetate adjusted to the correct acetyl value and then esterify some of the available hydroxyls with phthalic acid. The type of product required was one in which the phthalic acid was combined through only one carboxyl group, so that the reaction mixture used was pyridine and phthalic anhydride.[18] If pyridine is used with phthalyl chloride, or phthalic anhydride with a mineral acid catalyst, there is certain to be cross-linking of the cellulose molecules and production of insoluble esters.

Cellulose acetate of about 49–50 per cent combined acetic acid content was dispersed in a mixture of methylene chloride and pyridine and treated with about twice the amount of phthalic anhydride required to esterify the one available hydroxyl group per unit. The reaction mixture was maintained at about 50° and after several days the ester was precipitated by pouring into hot acidified water. The pyridine was thus neutralized, the methylene dichloride vaporized and the ester was liberated. The white fibrous material, after washing and drying, contained approximately 2·0 acetyl groups, 0·5 phthalyl, and 0·5 hydroxyl groups per glucose unit. This represents 26 per cent combined phthalic acid, and the mixed ester is soluble in benzene-alcohol, a variety of ketones and esters and, after conversion to the sodium salt, in dilute sodium hydroxide or carbonate.

A recent advance in this art is that the phthalylation can now be carried out on a secondary acetate dissolved in acetic acid containing the excess of phthalic anhydride, provided that sodium acetate is used as the catalyst.[19] Starting with an acetate of appropriately low acetyl value, mixed esters with up to 30 per cent combined phthalic acid are readily obtained, and the phthalic is combined through only 1 carboxyl group. Thus, it is no longer essential to use pyridine. An interesting fact which came out of the recent work was that the final extent of phthalylation is dependent on the temperature employed. Reaction proceeds most rapidly at high temperatures, say, at 95°, but does not proceed so far as at 40°. The best method, then, is to phthalylate for a few hours at 95° and then to cool to 40° and maintain at that level for, say, 24 hr. By this means there may be a gain of some 2–3 per cent in phthalic acid content.

These acid esters can be used as water-insoluble coatings which can later be removed by swabbing with dilute alkali. Claims have been made for the usefulness of these compounds, but they have never become of commercial importance.

As an example of a mixed ester of cellulose containing an unsaturated linkage, cellulose acetate–crotonate is chosen for description. The main idea in introducing an unsaturated group into the cellulose ester is to have present a group which will polymerize. Crotonic acid is reasonably cheap and is not so active as to give rise to uncontrolled cross-linking. It is essential that the further polymerization should take place only at the desired stage during use of the ester. The ester may be made by a two-stage process involving reaction with crotonic anhydride prior to acetylation with acetic anhydride. The catalyst used is 2·5 per cent concentrated sulphuric acid based on the weight of cellulose, and a convenient solvent to use is nitromethane; when prepared in this way, and when followed by a short hydrolysis stage, acetate–crotonates having up to 1·8 crotonyl groups and 0·3 hydroxyl groups per glucose unit may be obtained. Work of a similar kind has been carried out recently in Germany and techniques have been reported for the preparation of a complete series of esters from tricrotonate down to triesters of low crotonyl content.[20] The cellulose used was specially pre-treated cotton linters, and the solvent medium was methylene dichloride, with perchloric acid as the catalyst. A smooth and quick esterification with the anhydride only was secured with the temperature rising to about 40°, and no polymerization of the crotonic anhydride or acid was experienced. Esters containing more than 0·5 crotonyl group were soluble in acetone and in general the triesters were soluble in a wide range of organic solvents.

The products of 1·5 or higher crotonyl substitution cross-linked easily on heating for some hours at 100°, or even on long standing at room temperature. The polymerization could be induced also and rather more quickly by peroxides and other addition polymerization initiators.

Cross-linking results in complete loss of solubility and of fusibility. Any textile advantage to be gained from this has so far been offset by the poor tensile strength and extensibility of cross-linked fibres.

The acetate–crotonates react readily with halogens, and simple addition of bromine has resulted in cellulose acetate–dibromobutyrates. These are of academic rather than practical interest, but they are non-inflammable.

More interesting, however, is the cross-linking of the crotonic esters with diamines. Addition of, for example, hexamethylene diamine to a solution of acetate–crotonate results very rapidly in gelling and precipitation of an insoluble, cross-linked, and nitrogen-containing compound. Alternatively, films or fabrics made from the soluble acetate–crotonate may be treated by impregnating with an aqueous solution of the diamine. Although the alkalinity would be sufficient to cause some saponification of cellulose acetate, the acetate–crotonates suffer only cross-linking, even when the crotonyl substitution is as low as 0·2. Indeed, from the point of view of theoretical uptake of the diamine, the acetate–crotonates of lowest crotonyl content are by far the most efficient (Table 3).

Table 3: Combination of hexamethylene diamine with cellulose acetate–crotonates

Product	Combined nitrogen		
	Theory	Found	Percentage of theory
2·5 Crotonate–0·3 Acetate ...	7·02	1·64	23·4
1·5 Crotonate–1·5 Acetate ...	5·07	2·10	39·6
0·5 Crotonate–2·5 Acetate ...	2·21	1·21	54·8
0·125 Crotonate–2·875 Acetate ...	0·58	0·50	86·2

Cross-linking in this way leads to insolubility and elevation of melting point but, in contrast to short cross-links formed by addition polymerization of the crotonyl groups, the lengthy polymethylene bridges can, if present in small amount, confer a very attractive flexibility and extensibility on the product. Moreover, the presence of combined nitrogen in amount no greater than 0·5 per cent confers on films and fibres a good affinity for the type of dyestuffs used for dyeing wool.

Cellulose acetate–sorbates, produced in America, are the only unsaturated mixed esters advertised as available commercially.

*Cellulose Toluene-*p*-sulphonates and Methanesulphonates*

These are products of the reactions of cellulose with toluene-*p*-sulphonyl chloride and methanesulphonyl chloride respectively. Those in which toluene-*p*-sulphonyl chloride has been used are possibly best known as mixed esters, since the principal interest in the use of these esterifying agents has lain in studies of the configuration of cellulose derivatives. Cramer and Purves[21] have shown that the primary hydroxyl group of cellulose reacts much more readil than do the secondary hydroxyl groups with toluene-*p*-sulphony chloride, and that only the toluene-*p*-sulphonyl groups in th primary position react with sodium iodide in acetone to be replace by iodine.

By esterifying the residual hydroxyl groups in either cellulos esters or ethers, with sufficient time at least for all free primar hydroxyl groups to react, and then replacing the primary toluene-*p*-sulphonyl groups, it becomes possible to say what proportions of th original free hydroxyls were primary and secondary. Exploratio of this technique showed that first-order rate constants for tosylatio of unsubstituted hydroxyls were roughly in the ratio of 1·0 for th 2-position, 0·06 for the 3-position and 10·0 for the 6-position.[21] I was therefore not difficult to devise conditions in which all th primary hydroxyls in the 6-position were tosylated, with reaction o only a minor proportion of the secondaries, mostly in the 2-position.

The intermediate toluene-*p*-sulphonyl mixed esters or ester–ethers are of little interest in themselves. Some further activity has recently been shown in the field of methanesulphonyl cellulose, where methods have been developed for preparing esters of much higher degree of substitution than had hitherto been reached. Owing, it is thought, to the smaller size of the methanesulphonyl group when compared with the toluene-*p*-sulphonyl, methanesulphonyl chloride is a more effective esterifying agent, there being less steric hindrance to its introduction. With properly prepared alkali cellulose and methanesulphonyl chloride in pyridine, a substantially trisubstituted cellulose methanesulphonate can be prepared.[22] Some chlorination cannot apparently be avoided, but in esters where the degree of substitution was 2·97, methanesulphonyl groups accounted for 2·70 and chlorine for 0·27.

Cellulose methanesulphonates of high degree of substitution are insoluble in most solvents, and solubility can only be achieved by drastic reduction of the degree of polymerization. Mixed esters with acetic acid are, however, soluble in a number of common solvents.

This technique of methanesulphonation has recently been extended to provide flame-resistant cotton or viscose materials.[23] Methanesulphonyl chloride does preferentially react at the primar hydroxyl; and it has been held that, to retard flammability in cellulosic materials, opportunity for formation of *laevo*glucosan by degradation must be prevented.[23,24] This can be achieved by removal of the labile hydrogen of the primary hydroxyl group, either by oxidation at the primary alcohol to carboxyl or by blocking it with, say, a sulphonyl group. In effect, both method lower the flammability, but in the case of the methanesulphonated products there remains an undesirable after-glow. To improve on this, methods have now been worked out for replacing some or all of the methanesulphonyl groups by halogen atoms, by heating the sulphonated fabric under pressure with aqueous alkali-metal halide in the presence of barium carbonate. Bromine and iodine are the most effective substituents; the brominated or iodinated cellulose carries not only the flame-retardant property of the halogen itself, but from its structure has a greatly reduced capacity for formation of *laevo*glucosan when decomposed by heat. This process, however, is not known to have progressed beyond the experimental stage.

Fibrous Cellulose Esters

Clarke and Malm[15] described the ' impelling ' action of chloracetic anhydride for the acetylation of cellulose by other acids as long ago as 1932, but later Bourne and co-workers[16] drew attention to the use of trifluoroacetic anhydride in impelling other acids into combination with cellulose. By such means a variety of cellulose esters were produced by the solution method without any combination whatsoever of the trifluoroacid with the cellulose.

Recent work has been devoted to partial esterification of cotton cellulose in an inert medium, such as benzene, with scoured cotton yarns as raw material. The results confirm the effectiveness of this anhydride, and show that a wide variety of acids may be brought into combination with the cellulose. Among the partial esters prepared were the cyano-acetates, oleates, sorbates, cinnamates, *iso*butyrates, and others from acids which normally esterify very slowly.[25]

CELLULOSE ETHERS

The principal cellulose ethers are the methyl, ethyl, hydroxyethyl, and carboxymethyl celluloses, the first three of which, at least, are quite long established, and also mixed ethers designed to achieve properties not otherwise provided. The four specified are made by reacting cellulose, impregnated with caustic soda solution (soda cellulose), with either methyl chloride, ethyl chloride, ethylene oxide, or monochloracetic acid, depending on the ether required. For water-soluble ethers, it is generally necessary to use a concentration of at least 40 per cent caustic soda and to have present 3 or more molecules of hydroxide per glucose unit.

The introduction of alkyl or substituted alkyl groups into the cellulose, by pushing apart the cellulose chains, opens it up to penetration and solution by hydrolytic solvents. Methyl cellulose, for example, becomes water-soluble at a degree of substitution of 1·3, ethyl cellulose at 0·7, and sodium carboxymethyl cellulose at 0·5. It appears that the bulkier the substituent group, the lower is the degree of substitution required for solubility in water.

The properties of the cellulose ethers depend upon the degree of etherification. So far as methyl and ethyl celluloses are concerned, it should be made clear that methyl cellulose is made principally for water-soluble applications, while ethyl cellulose is almost wholly produced as a highly-substituted, water-insoluble material for conventional extrusion and injection moulding purposes. Something of the properties and application may be gained from the following description, which applies in particular to methyl cellulose but which, in many respects, would apply also to ethyl, hydroxyethyl, and to some mixed ethers.

Methyl Cellulose

The various commercial types and viscosity grades of methyl cellulose are mostly water-soluble, and are produced as powders or as white fibrous materials. Clear viscous solutions, up to 5 per cent in concentration, have the properties usually associated with protective colloids, and may be compared with the water-soluble natural products such as gum tragacanth, agar-agar, starch, and glue. The cellulose ethers are usually available, however, in a number of viscosity grades, thus permitting a wide choice of materials and working conditions.

Films obtained by drying methyl cellulose solutions are tough and flexible. They may be produced plasticized with glycerol or polyethylene glycols, sorbitol, or triethyl phosphate. The films may be rendered insoluble in water by incorporating formaldehyde, dimethylol–urea, water-soluble urea–formaldehyde and melamine–formaldehyde resin, or other suitable polyfunctional reagents. Stoving at temperatures around 100°–110° will be found to induce insolubility by cross-linking, due to reaction of the functional reagent with itself and with the available hydroxyls of the cellulose ether.

Methyl celluloses, of the water-soluble variety, are not appreciably thermoplastic and they have a charring point of about 220°–230°. They dissolve readily in cold water, but coagulate gradually if the temperature is raised. On cooling, complete solubility returns, and they thus behave in the reverse manner to other hydrophilic colloids such as agar-agar and gelatine, which gel on cooling.

The poor thermoplastic properties of commercial methyl celluloses are probably due to the fact that the methoxy groups are not sufficiently bulky to give internal plasticization, and consequently methyl cellulose does not lend itself readily to shaping by extrusion. It finds application as a protective colloid or dispersing agent in paints, printing inks, cosmetic creams, textile printing and finishing, pharmaceutical emulsions, and shoe creams. It is also used as a thickener in such products as paint removers, toothpastes, edible creams, detergents, sauces, foamed confectionery, and salad dressings. It also has extensive use as a binder in sizes and distempers, as an adhesive, and as an oil-resistant film-forming material. It will be apparent from what has been said that methyl cellulose is physiologically inert to a degree that permits its use in food and drugs.

At the rather higher degrees of substitution, methyl cellulose may be soluble not only in water but also in certain mixtures of organic solvents, for example in methylene dichloride-alcohol.

Methyl cellulose generally has from 27 per cent upwards of combined methoxyl representing at least 1·6 methoxyl groups per glucose unit. Introduction of hydroxyethyl groups with the methyl, e.g. by successive reaction of soda cellulose with ethylene oxide and with methyl chloride, raises the temperature at which coagulation of aqueous solutions sets in, and the same effect is observed with ethyl hydroxyethyl ethers. Hydroxyethyl ethers, sometimes known as glycol cellulose, are so much more hydrophilic in nature, due to the fact that the number of hydroxyl groups per glucose unit remains undiminished, that they do not show coagulation as the temperature of an aqueous solution is raised. The hydroxyethyl ethers are used quite extensively to thicken and give body to emulsion paints.

Addition of ethylene oxide to soda cellulose has to be done under carefully controlled conditions if it be desired to favour the introduction of simple hydroxyethyl groups. However, the primary hydroxyethyl group readily reacts with more ethylene oxide so that there are always some polyethylene oxide side-chains built up.

Much work has been devoted to determining the exact disposition of the substituents on the anhydroglucose unit and along the cellulose chain.[26] The exact conditions of the etherification reaction are a dominating factor, but within the unit it is usual with methyl or ethyl celluloses to find substitution on primary and secondary hydroxyls in roughly the expected proportion of 1 to 2. It is important to note, however, that uniformity of substitution along the chain is probably poor, since, when special attempts are made to secure maximum uniformity, water solubility is achieved with a much lower degree of substitution than is usual in commercial materials.

Carboxymethyl Cellulose

Commercially available water-soluble sodium salts of carboxymethyl cellulose are known as C.M.C. The free acid form is not very soluble in water. C.M.C. is made usually in degrees of substitution from 0·3 to 1·2, and is usually prepared from soda cellulose and sodium monochloroacetate. The industrial product, generally containing some of the by-products, sodium chloride and sodium glycollate, is a cream-coloured powder, with a charring point above 210°. It is soluble in both cold and hot water, and can be cast into films from aqueous solutions. However, for many applications, the films are too water-sensitive. C.M.C. has found considerable outlet as a wallpaper paste, and as a constituent of detergents.

Benzyl Cellulose

This is not produced commercially in Britain or the United States, but has found particular favour in Germany for coating, plastics, and lacquer applications. It is a white granular material, with the rather low melting point of 100°–150°, due to the effect of the bulky side-chains. Preparation is carried out using soda cellulose and benzyl chloride. It is soluble mainly in organic liquids, and its internally plasticized nature obviates the need for added plasticizer in plastics applications. However, its heat and light stability are low and with its general softness it is not very attractive from a commercial point of view.

Cyano-ethyl Cellulose

There are certain ethers of interest, which are made by a Michael-type addition to the cellulose hydroxyl groups, of compounds containing an activated double bond. Among many other reactions investigated, Bruson and Riener[27] showed that acrylonitrile, for example, will in the presence of alkaline catalysts add to alcohol groups, thus

$$\mathrm{ROH} + \mathrm{CH_2} = \mathrm{CHCN} \rightarrow \mathrm{R.O.CH_2.CH_2.CN}$$

Acrylonitrile will cyano-ethylate the hydroxyl groups of cellulose,[28] the degree to which it does so depending on the reaction conditions, especially on temperature, concentration of alkali, and time of reaction. Cyano-ethyl groups are rather easily hydrolysed to carboxyethyl and care has therefore to be taken to avoid this. Many examples of these ethers have been prepared by treating xanthate solutions with acrylonitrile. MacGregor[29] has claimed by such means to produce alkali-soluble and water-soluble ethers of degree of substitution 0·3 and 0·7–1·0, respectively. It appears that from a xanthate source the preparation of organo-soluble ethers is difficult, since the xanthate group blocks formation of a highly substituted ether. With 2·5–3·0 cyano-ethyl groups, solubility in organic solvents can, however, be obtained. Perhaps, once again, more interest lies in the heterogeneous cyano-ethylation of cotton yarns and fabrics, studied by the Southern Regional Research Laboratory, New Orleans, Louisiana, U.S.A., where experiments have shown that almost any degree of substitution up to 2·0 may be achieved without destroying the fibrous form of the cotton goods. Up to a degree of substitution of 2·7 the products were unaffected by both water and organic solvents, and this, in the light of earlier statements, must be attributed largely to the high degree of polymerization of the cotton.[30]

One procedure used is to soak the material in 2 per cent sodium hydroxide solution and squeeze so that the amount of solution absorbed is equal to 50–52 per cent of the original weight of the fabric. The roll of cloth is then subjected to a circulating stream of acrylonitrile at 55° for about 1·5 hr. After draining and neutralizing with acetic acid, the cloth is washed and dried. It contains about 4·3 per cent of combined nitrogen, which represents a degree of substitution of 0·6, sufficient to achieve enhanced heat, rot, and abrasion resistance without undue change in strength and elasticity.

Ethers from Other Activated Olefine Compounds

Workers at the Southern Regional Research Laboratory, on the basis of some earlier work by other investigators,[31] extended their study of cotton modifications to the use of other unsaturated compounds.[32] They do not claim much more than that this Michael-type reaction may have rather wide application in producing useful cellulose ethers, but they did find that acrylamide reacted readily with cotton cellulose to give a carbamoyl-ethylated product, that is, a cellulose ether having groups of the type $O.CH_2.CH_2.CO.NH_2$. This was accomplished by impregnating cotton fabric with an aqueous solution containing 50 per cent acrylamide and 4 per cent sodium hydroxide in such manner that the weight of liquor absorbed was equal to 80 per cent of the original weight of the fabric. The material was then stoved for 4 min at 135°, washed, and dried. Breaking strength of the fabric was not significantly affected, rot resistance, while improved, was not very good, but heat resistance at 160° was much better than in the original fabric. The material stood up well to rather vigorous laundering conditions, there being little loss of the 2·4 per cent combined nitrogen, which is equivalent to about 1 carbamoyl-ethyl group in every 3 glucose units.

Among a large number of other activated olefine compound tried, only acrylic acid and maleic acid could be said to have any appreciable activity towards the cellulose. Crotonaldehyde gave an interesting orange-coloured derivative of cellulose (Cellulose–$O.CH(CH_3)CH_2.CHO$) which had quite marked reducing power when boiled in Fehling's solution. Degree of substitution was however, small, there being but 1 aldehyde group per 30 glucose units.

CELLULOSE THIOURETHANES

It is 30 years since Lilienfeld first prepared an alkali-soluble phenyl thiourethane of cellulose, but recent work by the American Viscose Corporation uses a different approach and has provided results which suggest that these urethanes have commercial possibilities. It is fairly easy to introduce a urethane linkage into cellulose, or at least into derivatives that still possess some available hydroxyl groups. Di*iso*cyanates, for example, cross-link secondary cellulose acetate quite readily between hydroxyls on adjacent chains, producing a urethane structure as below.

$$\begin{gathered}\text{—OH}\quad \text{OCN.(CH}_2)_n\text{.NCO}\quad \text{HO—}\\ \downarrow \\ \text{—O.CO.NH.(CH}_2)_n\text{.NH.CO.O—}\end{gathered}$$

Doubtless di*iso*thiocyanates would react similarly, but in the products under discussion the thiourethane groups are formed quite differently, and linear, not cross-linked, products are produced. Sodium cellulose xanthate can be stabilized against hydrolysis apparently by conversion to zinc cellulose xanthate. This is made into a slurry with water and is treated with aniline whereby the following series of reaction steps is completed.

$$\begin{tikzcd}
2\ \text{Cell—O—C(S)SNa} + \text{Zn}^{2+} \arrow[r] & \text{Cell—O—C(S).S.Zn.S.(S)C—O—Cell.} \arrow[d, "2H_2O"] \\
2\ \text{Cell—O—C(S).NH.C}_6\text{H}_5 & 2\ \text{Cell—O—C(S).SH} + \text{Zn(OH)}_2 \arrow[l, "C_6H_5NH_2"']
\end{tikzcd}$$

Allewelt, of the American Viscose Corporation, describes how such a derivative has been formed into a spinnable solution containing 8·5 per cent of the cellulose phenyl-thiourethane and 3 per cent caustic soda, and records[33] a spinning process in which the coagulant is 30 per cent aqueous ammonium sulphate at 5°. Yarns of acceptable strength (2 g/denier, i.e. of the same order as normal viscose rayon) were obtained, but, at least in the initial experiments, the elongation at break of 6–9 per cent was much too low for textile use.

The new material resembles wool in having both sulphur and nitrogen in the molecule. The sulphur content is 5·5 per cent and the nitrogen 3·4 per cent and the material has excellent resistance to microbiological attack, being much superior to viscose in this respect. It has less moisture absorption than either viscose or wool, but is not so hydrophobic as acetate. The cellulose phenyl thiourethanes, like wool, suffer decomposition on prolonged boiling in water, but the break-up is confined to the urethane substituent, the cellulose chain undergoing no appreciable degradation. Unfortunately, this material in fibre form has very poor stability to ultraviolet light.

CONCLUSION

In this brief survey of a very large field of work it has been possible to note only some of the principal activities. So far as the future is concerned, modifications of esterification techniques foreshadow the spinning of cellulose esters direct from the esterification mix, so that cellulose will enter at one end and filament yarn will be extruded at the other. The trend towards triacetate in the cellulose acetate field will, if it continues, help towards such continuity of process.

Modern large-scale developments in aliphatic chemistry make probable the day when other aliphatic acids, particularly propionic, may be as cheap as acetic acid, and thus cellulose propionates, or other mixed esters, may more readily find acceptance in plastics applications.

It is along these two lines, at least so far as the European market is concerned, that development is most likely. All expectation of progress in the field of cellulose derivatives must, however, be judged in the light of the strong challenge from the host of fully synthetic textile and plastics products.

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8

MECHANICAL PROPERTIES OF CELLULOSE AND CELLULOSE DERIVATIVES

INTRODUCTION

Previous papers have dealt with the chemistry, molecular structure, crystal structure, and fine structure of cellulose and its derivatives. It is now proposed to deal with some aspects of the mechanical properties of cellulose and its derivatives and their relationship to molecular structure.

The high degree of polymerization and the linear nature of the macromolecules of cellulose lead to the formation of fibrous structures which by virtue of their crystallinity and molecular orientation provide a basic material with excellent mechanical properties which can be used to advantage in the textile, paper, and plastics industries. What follows is concerned mainly with the mechanical properties of those cellulose fibres which are used in the textile industries.

One of the most important of the mechanical properties is the tensile strength of the fibre. Apart from dimensional characteristics such as length and fineness, fibre strength has been regarded as a good index of fibre quality and much attention has been given to the work of measuring this property. Although fibre strength will be dealt with, the mechanism of deformation and the more fundamental properties of creep, relaxation, dynamic modulus, and internal friction will be considered first.

MECHANISM OF DEFORMATION

A brief account of the mechanism of deformation[1,2,3] is given here in general terms before proceeding with the more detailed consideration of mechanical properties. The essential feature of the structure of cellulose on which its mechanical properties depend is the network structure formed by bundles of chain molecules, in the noncrystalline regions, which link together the crystallites. These crystallites act like ‘ spot welds ’ and although they contribute to the mechanical behaviour, the characteristic mechanical properties are determined mainly by bond stretching and chain straightening in the non-crystalline regions of the cellulose.

The network can be considered to consist of chains of bond joining one point to another. Some sections of such chains wil consist of segments of chain-molecules; others will consist of bond between different molecules or molecular segments, and these wil in general be of a weaker type than the others. Only the intermolecular bonds will contribute significantly, by actual stretching to the extension of the fibre, since the force constants of the covalent bonds are so high that extension of the chain molecules by bond deformation within the chain molecule itself is very small. When subjected to steadily increasing deformation, the stress on a bond rises to a maximum and then decreases; the bond ' breaks ' when the deformation reaches a value corresponding to this maximum stress. Bonds of the hydrogen or van der Waals' type extend by something between 10 per cent and 20 per cent; thus, an orientated chain of hydrogen bonds can be expected to have a potential reversible elasticity of not more than 20 per cent, which will be reduced if the chain contains other stronger bonds.

More than one chain of bonds will join any two points not too close together and in the unstretched state these chains will in general have different lengths and configurations. Before a given bond is stretched, it must be orientated in the direction of the applied strain and the necessary orientation will be induced via the shorter chains. This introduces the possibility of bond breakdown with reformation when the strain is released through the elasticity of the longer chains of unbroken bonds. If a sufficiently drastic degree of breakdown occurs in the bond network, or newly formed bonds are sufficiently stable, permanent or temporary set will occur. If the newly formed bonds are not stable, the residual unbroken bonds in the network will tend to pull the system back to its original state, assisted by energy of thermal agitation, and the recovery will be time dependent, i.e. creep recovery will occur. When a fibre has been subjected to strain for a period of time and the strain is removed, complete recovery requires a considerable time. If the fibre is held at some length greater than that which it would ultimately assume, a recovery of tension is observed. This is a consequence of the same events which produce creep recovery.

The structural changes accompanying stretching can best be followed by X-ray and birefringence measurements. X-rays enable the molecular orientation of the crystallites to be measured whilst optical data give the orientation of the chain molecules in both crystalline and non-crystalline regions. It has been shown by

Hermans (ref. 1, pp. 261, 455) that when the degree of orientation is low, the orientation in the non-crystalline regions is lower than that of the crystallites; when the degree of orientation is high, the orientation of the chain molecules in the non-crystalline regions approaches, and may equal, that of the crystallites. The highly ordered regions are orientated during the initial stretching of the fibre.

The relation between orientation and extension has been analysed mathematically and tested experimentally by Hermans et al.[4] Two special cases, representing extremes of polymer structure, were worked out in detail. In the first case, the crystallites were considered to be rigid, rod-like particles of definite axial ratio embedded in a viscous liquid; in the second case, the model was a network of bundles of chain molecules connected by flexible joints. Neither system was completely successful in explaining the mechanism of deformation of actual cellulose fibres or films, which in fact behave as though they are a mixture of the two cases. Specimens of cellulose in a highly swollen state approach more nearly to the first case while dry specimens approach the behaviour of the model assumed in the second case. Progress was made by Hermans[5] who assumed a network of molecular chains kinked at random with crystalline regions as junction points, and he worked out a theory similar to that used to explain rubber-like elasticity, but modified this theory to take into account the shorter chain lengths between junction points in cellulose. This theory helps to explain the relation between tensile stress and orientation (see ref. 1, p. 483 et seq.). Depending on the degree of swelling of the cellulose and its temperature, the mechanism of deformation will involve changes in both internal energy and entropy; further consideration is given to this in the section on stress–strain curves.

CREEP UNDER CONSTANT LOAD

A simple experiment that promises to provide a link between the mechanical properties and structure of fibres consists in applying a constant load to a fibre or filament and observing the way in which the extension depends on the time of application of the load and the manner and extent of recovery after removal of the load. As a typical example of the behaviour of a cellulose fibre, consider the effect of the magnitude of the load on the creep of viscose rayon. When the load is applied, there occurs a rapid initial extension followed by a further extension that proceeds at a steadily decreasing rate until either an apparent equilibrium between load and extension is reached or, if the load is high enough, the rate of cree remains measurable and the fibre breaks. By plotting the extensio against the logarithm of time, the manner in which the initial rapi creep takes place can be studied and the continuing nature of th creep process over long periods of time becomes apparent.

The amount of time-dependent extension is a large fraction of th total extension over a period of a day. When the load is remove at the end of this time, there is an immediate elastic recover followed by a time-dependent recovery such that after 24 h recovery, the fibre shows considerable ' set ', all of which can b recovered by swelling the fibre in warm water provided the extensio has not exceeded a certain value: above this value, permanent se occurs.

When the creep data are plotted as strain–stress against log(time the curves shown in Figure 1 are obtained. They show that withi the time range of the experiments, the amount of creep is relativel greatest for intermediate loads and the individual curves appear t be parts of a common sigmoidal curve which shifts to shorter time as the load increases, in much the same way that the sigmoida creep curve at constant load moves to shorter times as (i) th relative humidity is increased,[6] and (ii) the temperature is increased.

It is instructive to analyse a creep curve for viscose rayon take with a constant load of such a magnitude that it gives a sigmoida creep–log(time) curve within a range of times that are convenien for observation, say, from a fraction of a second to a few days. Th equation used by Mark and Press[8] to represent the creep of thei viscose rayon was

$$e\ (\%) = 0{\cdot}6 + 1{\cdot}9 + 1{\cdot}0\ (1 - \exp(-t/50)) + 0{\cdot}9 \log_{10}\ (t + 1)$$

It was derived from the experimental data in the following wa Dynamic modulus measurements of very short duration gave value of 0·6 per cent for the instantaneous extension. Between 0·0 and 1 sec, there was a further 1·9 per cent extension which is ver slightly retarded elastic extension with a retardation time of les than a second. Beyond 120 sec the creep is mainly logarithmic an is represented by the last term of the above equation. The remainde of the right-hand side of the equation represents a delayed deformation with a retardation time of 50 sec.

The molecular interpretation of the terms in the above equatio is that the first term represents the stretching of intermolecul bonds (e.g. hydrogen bonds between the chain molecules). Th fast elastic response with a range of about 2 per cent probabl represents rubber-like elasticity of randomly kinked and curled-u chains in the non-crystalline regions. Following this comes a considerably retarded elasticity with a retardation time of 50 sec, representing the displacement of larger flow-units consisting of bundles of chain molecules, and finally some crystallization occurs.

Let us now examine how this general interpretation of the sigmoidal creep curve observed for a fixed stress, humidity and temperature fits in with the observations that very little creep occurs at low stress, humidity and temperature, but that the amount of creep increases at higher stresses, humidities and temperatures. Low stresses will produce only small deformations so that most of the linkages between the molecules in the non-crystalline regions are strained elastically, i.e. the strain energy is stored and little is dissipated by the internal friction of molecular flow. After a sufficiently long time, the energy of thermal agitation produces a measurable amount of flow and the creep becomes easily measurable. At low temperature and low humidity, the modulus of deformation is high, so that a given stress produces only small deformations which will be mainly elastic in nature. As the stress or humidity increase, the average modulus of deformation decreases and the combination of strain energy and thermal agitation is sufficient to cause considerable rupture of the intermolecular bonds in the non-crystalline regions and relatively large segments of molecules

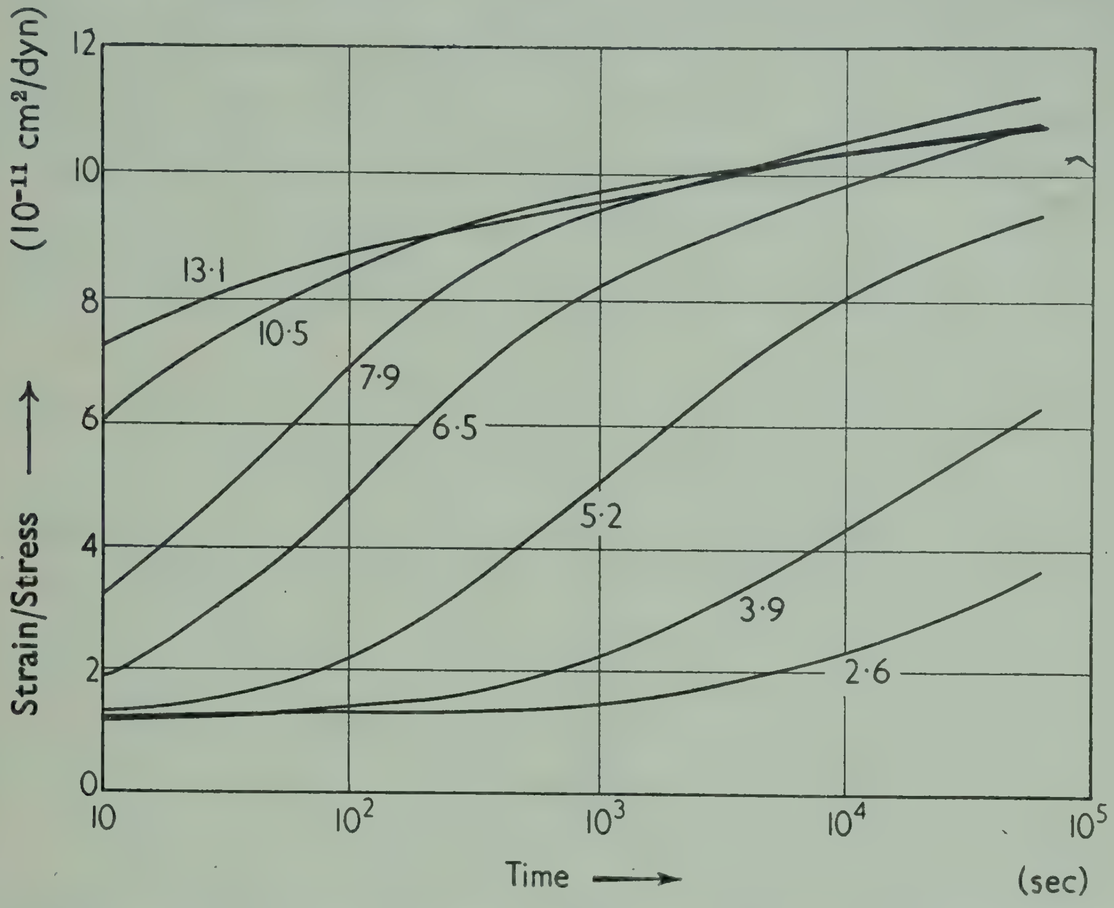


Figure 1. Creep curves for viscose rayon[54] *at 60 per cent R.H. and 21° for a series of constant loads given in units of* 10^{-11} *cm*^{2}*/dyn*

flow with respect to their neighbours so that the amount of creep becomes large. Increase of temperature at a given stress and humidity has a similar effect by virtue of the increased therma agitation which speeds up the flow of molecular segments. At high stresses or humidities, the amount of creep observed in a time interval of say 10–10^4 sec is small compared with the total deformation because most of the movement of molecular segments has already taken place before observations have started.

Although viscose rayon has been taken as an example, the creep behaviour described is typical of many linear high polymers including the natural cellulose fibres, although with these fibres, the modulus of deformation is higher and the changes of this quantity with stress and humidity are smaller so that the magnitude of the creep is less.

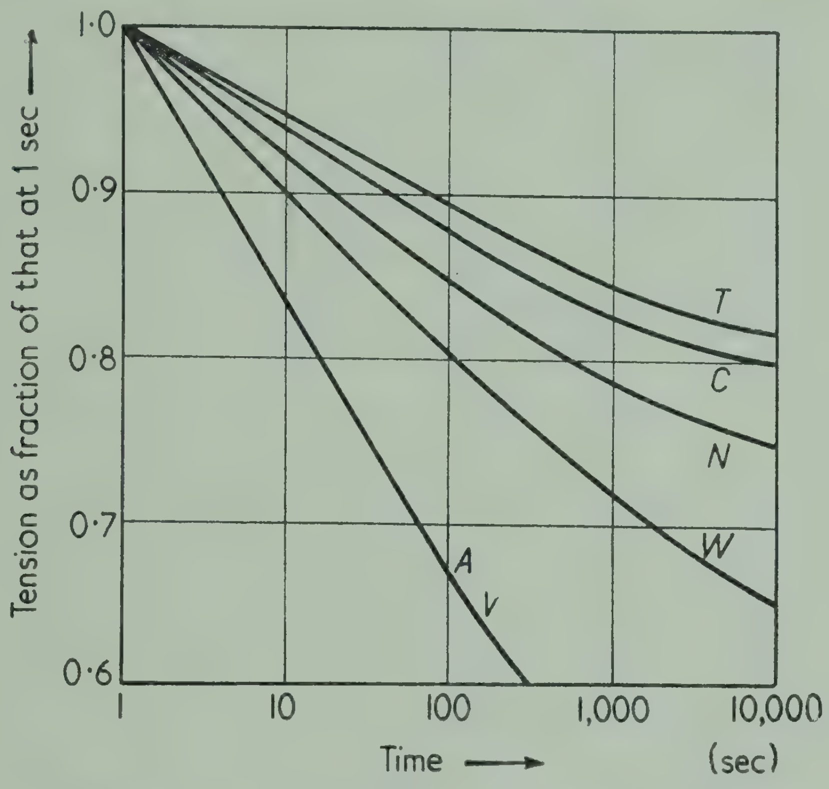
STRESS RELAXATION AT CONSTANT STRAIN

During a creep test under constant load, the area of cross-section decreases as extension proceeds so that the stress increases. This effect becomes important if the total deformation is large. In a stress-relaxation test in which the fibre is quickly extended by a certain amount and then held at constant length, the geometry of the strained fibre remains substantially unchanged and quantitative theoretical interpretation is simpler. Moreover, the externally observed effect represents more closely what is taking place within the fibre in both creep and relaxation experiments, namely, a relaxation of the internal stress on segments of the long chain molecules.

The relative rates of relaxation of a number of different fibres are shown in Figure 2 in which the tension expressed as a fraction of that existing 1 sec after rapid extension is plotted against the logarithm of the time. The constant extension used was 5 per cent in all cases; this enables a fair comparison to be made since the relative decrease in stress is little affected by the magnitude of the extension over a range from 2–10 per cent with most fibres.

The cellulose fibres are represented by cotton, viscose rayon, and cellulose acetate fibre. It will be observed that a considerable difference in rate of relaxation exists,[9] the relaxation of viscose and cellulose acetate being rapid and that of cotton slow. This is what one might expect from the fine structure of these fibres, the native cellulose having the smallest fraction of non-crystalline material with correspondingly fewer linkages available for rupture and associated relaxation of stress. In passing it should be noted that whereas Terylene behaves in a similar way to cotton, wool shows a greater rate of relaxation than cotton but less than viscose rayon. The relaxation rate of nylon is intermediate between those of Terylene and wool.

Increase in temperature produces an increase in the rates of relaxation of all fibres. For example,[10] in passing from 25° to 160°, the slope of the relative stress–log(time) plot increases by 30 per cent for dry cotton and by 80 per cent for dry viscose rayon. Increase in humidity,[10–13] also increases the relative rate of relaxation of cellulose fibres, the increase being greatest with cellulose acetate.



T = Terylene;
C = cotton;
N = nylon;
W = wool;
A = cellulose acetate;
V = viscose rayon

Figure 2. Decay of tension for various fibres at 65 per cent R.H. and 25° for constant extension of 5 per cent

Typical curves showing the effect of increased extension on the relaxation of viscose rayon over a wide range of times from 0·1 sec to 1 day are shown in Figure 3. At low extensions the curve is concave to the log(time) axis but, above 2 per cent extension, the curve becomes slightly convex to this axis. A similar set of curves is obtained for cellulose acetate.

If the data for viscose rayon and cellulose acetate are replotted as stress divided by strain against the logarithm of the time, each curve thus obtained appears to be part of a common sigmoidal curve shifted to lower values of log t as the constant extension is increased. The composite curves in Figure 4 were obtained by translating each individual curve along the log t axis until a fit of the overlapping ends was obtained. These curves give the correct relation between stress/strain and log t for a constant extension o 1 per cent; to obtain the curves corresponding to higher constan extensions, a relation between extension and the amount of shif ($\triangle$ log t) is required. For cellulose acetate, for example, the relation was found to be approximately $\triangle \log t = 12 \log e$, where e is the extension expressed as a percentage in seconds. The general characteristics of master curves in creep and relaxation are similar

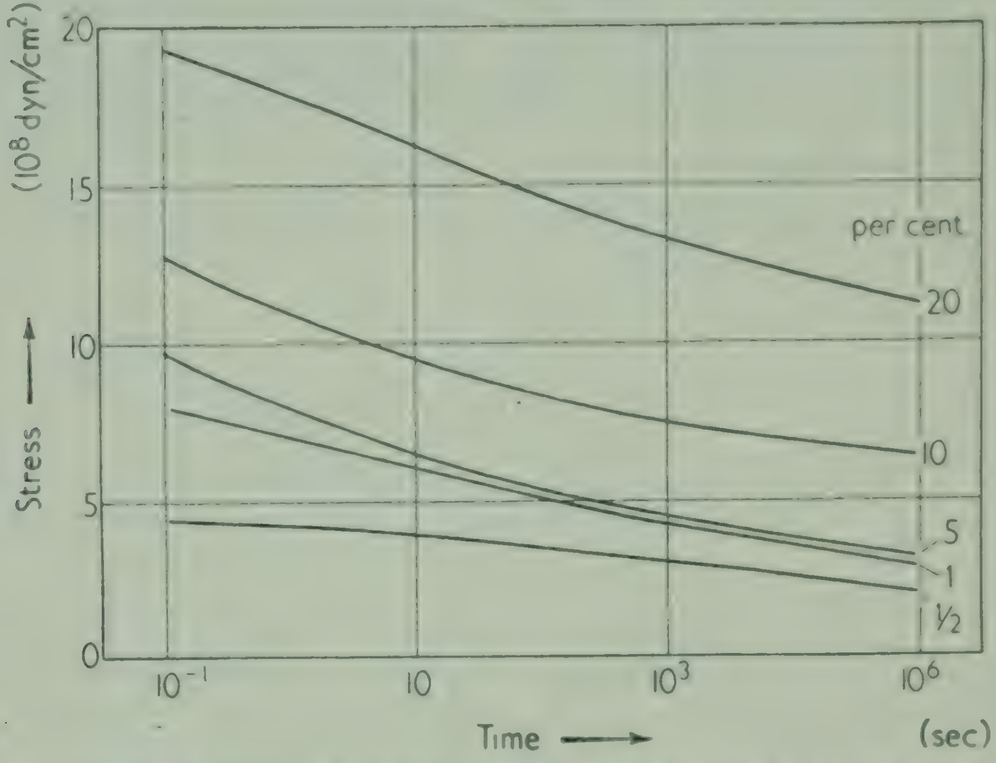


Figure 3. Relaxation of stress for viscose rayon at 65 per cent R.H. and 25° for constant extension from 0·5–20 per cent

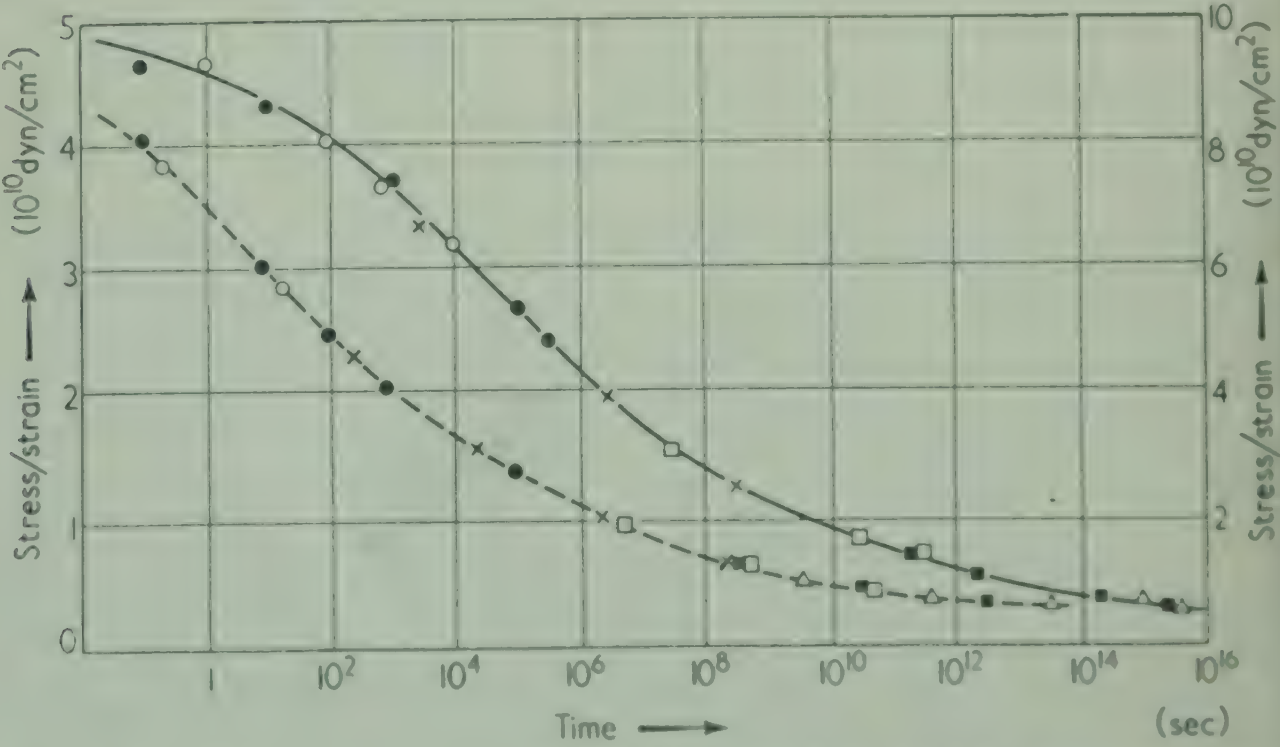


Figure 4. Composite stress-relaxation curves at 65 per cent R.H. and 25°. Continuous curve an left-hand scale refer to cellulose acetate. Dashed curve and right-hand scale refer to viscose ray

and Feltham[14] has claimed to account for this on the basis of a generalized statistical model. The significance of this composite curve is that it indicates that a broad distribution of relaxation times must be postulated in order to explain the relaxation behaviour of these fibres.

If it is assumed that the rate of decrease in stress is proportional to the stress on the fibre, then the stress (f_t) at any time t, after rapid stretching which produces an initial stress f_0 will be given by the relation $f_t = f_0 \exp(-t/\tau)$ where the relaxation time, τ, is the time in which the stress falls to 1/exp of its initial value. This relation gives a curve of stress vs log t in which almost all the stress decays over only two cycles of log t and which is linear over a 10-fold range of times from $\tau/5$ to 2τ. Decay of stress in cellulose fibres occurs over many cycles of log t and is linear with log t over a considerable range. This can be explained either (i) by assuming a distribution of relaxation times, $E(\tau)$, where $E(\tau)\,\mathrm{d}\tau$ specifies the contribution of those elements having relaxation times between τ and $\tau + \mathrm{d}\tau$ to the total instantaneous modulus E, so that, for a strain e_0,

$$f_t = e_0 \int_0^\infty E(\tau) \exp(-t/\tau).\mathrm{d}\tau$$

or (ii) by postulating a non-linear relation between the rate of flow of molecular segments and the internal stress, as has been done by Eyring[15] et al.

DYNAMIC MECHANICAL PROPERTIES

A knowledge of the dynamic mechanical properties of fibres must be added to the knowledge of static behaviour to establish a complete set of results on which to base a theoretical interpretation of the mechanical properties of fibres. The two quantities usually measured to characterize dynamic mechanical behaviour are elastic modulus and internal friction. These quantities are measured by subjecting the fibres to vibratory deformation, using either free or forced vibrations of small amplitude. Under these conditions one would expect the dynamic modulus to be independent of frequency and this is found to be so,[16,17] as demonstrated by the data in Table 1.

When energy losses arising from internal viscosity accompany mechanical deformation, a complex modulus can be used to express both the ordinary dynamic elastic modulus and the internal friction as a complex number in the following way. The force associated with viscous loss in a fibre may be expressed as $(\eta A/l)$ $\mathrm{d}x/\mathrm{d}t$ where η is a viscosity coefficient, A is the cross-sectional area of a fibre of length l extended by amount x. For a sinusoidally varying extension, $x = x_0 \exp j\omega t$, the force arising from interna friction becomes $j\omega\eta xA/l$. The total force associated with deformation arising from the combination of elastic and viscous effects i $f = (Ax/l)(E + j\eta\omega)$ where E is the dynamic modulus. For th static case, $f = (Ax/l)E_s$ where E_s is the static modulus. Fo equivalence of these two expressions, the static modulus E_s i replaced in the dynamic case by the complex modulus, $E^* =$ $E + j\eta\omega$. The loss tangent, tan δ, is given by the ratio $\eta\omega/E$ and is a convenient way of expressing the fraction of energy lost owing to internal friction, since the energy dissipated per cycle of vibration divided by the strain energy when the strain is a maximum, is simply 2π tan δ. The angle δ is the angle of phase lag between the impressed force and the resulting displacement. It will be seen from Table 1 that the loss tangent increases at low and high frequencies in the range covered. Since the dynamic modulus is constant, the internal friction ($\eta\omega$) varies with frequency in a similar manner to tan δ.

Table 1: Effect of frequency on dynamic properties at 20° and 65 per cent R.H.

Fibre	Static stress (10^8 dyn/cm^2)	Dynamic Young's modulus, (10^{10} dyn/cm^2)			Loss tangent, (tan δ)		
		1 c/s	10^2 c/s	10^5 c/s	1 c/s	10^2 c/s	10^5 c/s
Viscose rayon ...	6·3	13	14	14	0·05	0·03	0·045
Cuprammonium rayon ...	6·7	19	19	21	0·045	0·03	0·05
Cellulose acetate	4·1	5	5	6	0·03	0·03	0·04
Silk	5·1	16	15	15	0·02	0·02	0·025
Nylon 6 ...	4·1	5	5	6	0·09	0·075	0·10

An approximate distribution of relaxation times can be calculated from the internal friction, and for cellulose fibres this turns out to be relatively flat[17] and quite different from the 'wedge'-shaped distribution characteristic of rubber-like materials. Assuming a flat relaxation spectrum and definite upper and lower limits of relaxation time, it can be shown[18] that the slope of the stress–relaxation curve is related to the internal friction measured dynamically by the equation

$$-\mathrm{d}(f/e_0)/\mathrm{d}(\log t) = 4{\cdot}6\,(\eta\omega)/\pi$$

i.e. the negative slope of the relaxation curve is proportional to th internal friction measured at a frequency equal to the reciprocal of the time at which the slope of the relaxation curve is measured. Relaxation data[16] for a number of different fibres have been analysed[18,19] on this basis and an order of magnitude agreement between observed and calculated values of $\omega\eta$ has been obtained. Data for viscose and acetate are given in Table 2. The slopes of the relaxation curves taken at 0·5 and 1 per cent strain have been used to calculate $\omega\eta$, since the theory assumes that we are dealing with small strains. The first set of results refer to Dunell and Dillon's[16] relaxation curves which were linear from 10 to 1,000 sec and they pointed out that better agreement might be expected if the slope of the relaxation curve in the region of 0·01 sec were used. The second set of results refer to the author's[19] relaxation curves where the slope at 0·1 to 1 sec after stretching was used to correspond as nearly as practicable with the frequency of 50 to 100 c/s used in the dynamic determination of $\omega\eta$: also the relaxation curve used was that for a specimen which had been previously relaxed, to make the condition of the specimen comparable with that during the dynamic measurements which were made on a relaxed specimen. It will be noted from the ratio $\omega\eta_{\text{obs}}/\omega\eta_{\text{calc}}$ that very good agreement is then obtained for both viscose and acetate.

Table 2: Comparison of $\omega\eta$ from relaxation tests and dynamic measurements at 65 per cent R.H. and 20°

Fibre	Static strain (per cent)	Time to extend (sec)	$\omega\eta$ (10^8 dyn/cm^2) calc.	$\omega\eta$ (10^8 dyn/cm^2) obs.	$\frac{\omega\eta_{\text{obs.}}}{\omega\eta_{\text{calc.}}}$
Viscose	0·5	15	31	48	1·6
Acetate... ...	0·5	15	15	15	1·0
Viscose	0·5	0·003	44	42	0·9
	1·0	0·003	49		1·1
Acetate... ...	0·5	0·003	14	17	1·2
	1·0	0·003	17		1·0

The value of the dynamic modulus depends on the static strain of the specimen as shown for viscose rayon in the top curve of Figure 5. This modulus remains constant up to an extension of about 3·5 per cent in this case. For fibres with a higher degree of molecular orientation, the range of extension with constant dynamic modulus is smaller and the increase in dynamic modulus with static strain is greater.[20] Similar curves are obtained with cellulose acetate. In

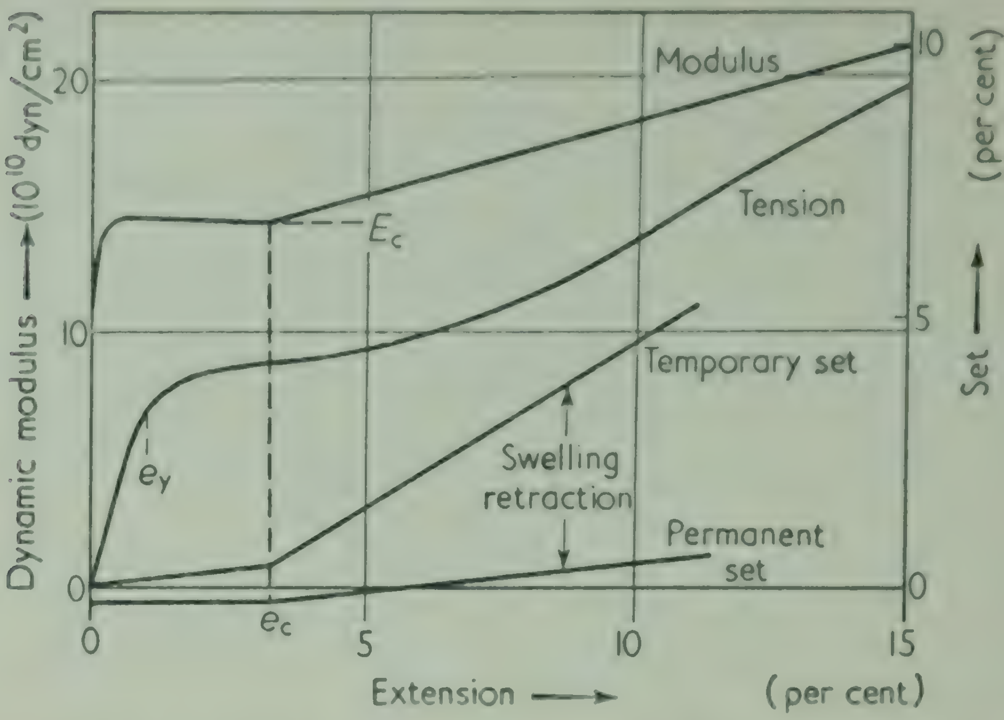


Figure 5. Corresponding curves for an ordinary viscose rayon showing dynamic Young's modulus, tension (arbitrary units) and components of deformation remaining after removal of tension[20]

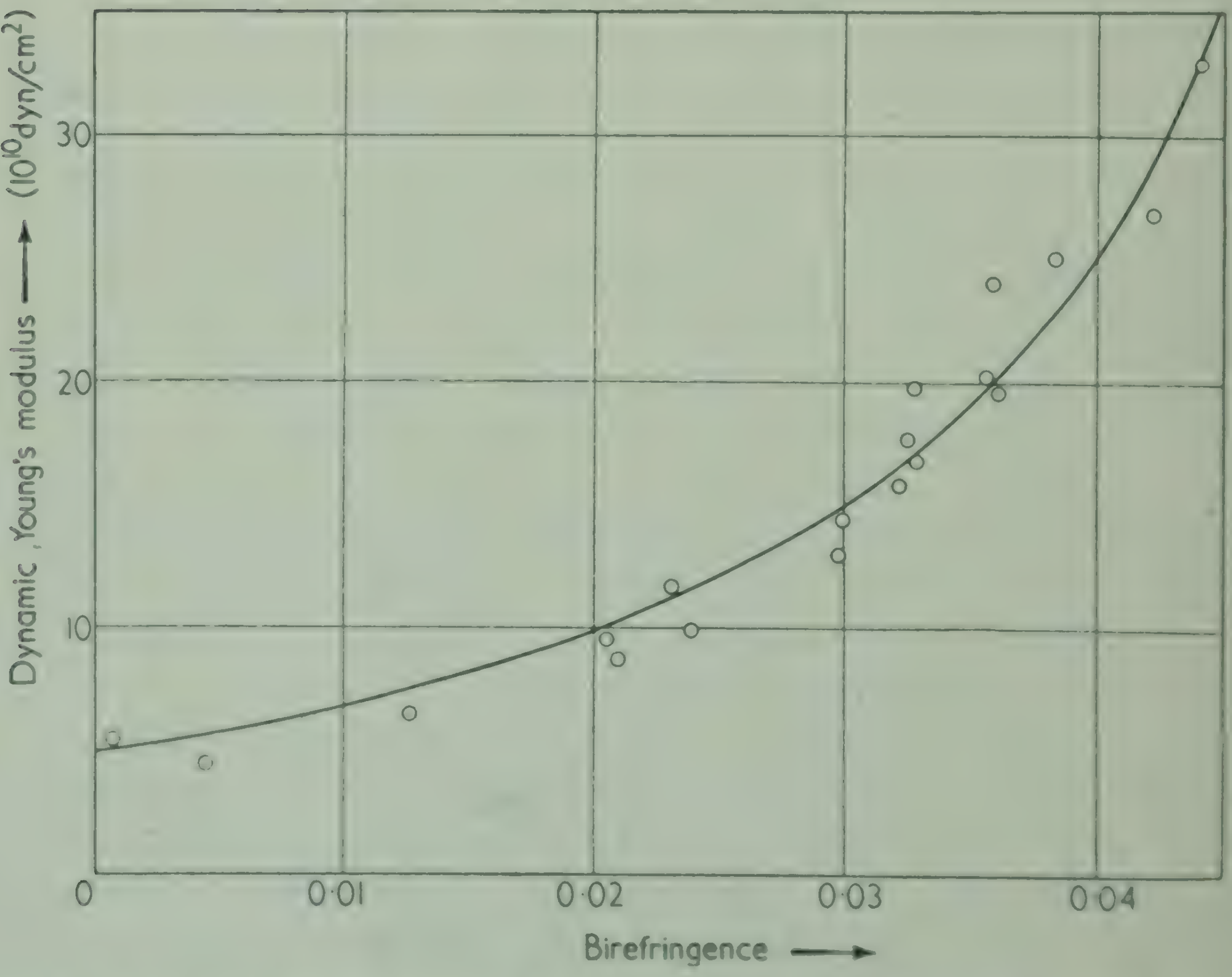


Figure 6. Correlation between dynamic Young's modulus and birefringence. Circles represent experimental points for normal viscose rayon filaments. Continuous line gives theoretical curve[55]

Figure 5, the stress–strain curve of the same sample is shown together with the components of deformation remaining after removal of tension and after swelling in water. Note that the value of the yield strain obtained from the stress–strain curve is appreciably less than the strain at which the dynamic modulus begins to increase. This means that the structure is permanently changed only by extensions beyond that at which the dynamic modulus begins to increase and not by any lower strain, even though the stress–strain curve has already passed the yield point (e_y). This is confirmed by the recovery data which show an appreciable increase in temporary set beyond e_c and, for the fibre after swelling recovery, no permanent set up to e_c.

The dynamic elastic modulus is a useful measure of the resistance to deformation of a fibre since it is substantially independent of time. De Vries[20] has worked out a theoretical relation between the dynamic modulus and birefringence of viscose rayons and the agreement with experimental observations is shown in Figure 6. The dynamic modulus increases from 5 to 35 × 10^{10} dyn/cm^2 as the birefringence increases zero to 0·045.

It is interesting to note that the tensile strength of viscose filaments, spun under a variety of conditions, is governed within fairly narrow limits by its birefringence, provided the birefringence is measured at the breaking point. As the birefringence increased from zero to 0·035, the tensile strength increased from 6 to 33 × 10^8 dyn/cm^2 (see ref. 1, p. 492).

THEORETICAL YOUNG'S MODULUS

The dynamic modulus is greater than the static modulus and is almost independent of frequency : it increases with the molecular orientation,[21] with reduction in moisture regain[22] and with reduction in temperature.[21]

The question arises whether the modulus could be calculated theoretically, e.g. from the intermolecular forces deduced from Raman and infra-red spectra. Only an upper limit to the modulus can be calculated because data which apply to the crystalline part of a cellulose fibre have to be used; knowledge of the non-crystalline regions is insufficient. Such a calculation was first made by Meyer and Lotmar[22] and later in its corrected form by Meredith.[23] The compliance of a glucose residue, i.e. the deformation per unit force, is the sum of the compliances due to stretching the C—C and C—O bonds and that due to distorting the valence angles. An average compliance of 2·2 × 10^{-6} cm/dyn (from Raman spectra of ethane and methyl alcohol) is assumed for these bonds and the application of simple trigonometry leads to a value for the total compliance o a glucose residue equal to $1{\cdot}76 \times 10^{-5}$ cm/dyn. Young's modulu (E) is defined as the ratio of tensile stress to strain or $E = (f/A)$, $(\triangle l/l)$ where f is the force on cross-sectional area A and $\triangle l$ is the elongation based on a length l. The compliance of the glucose residue $C = \triangle l/f$ and taking the area of cross-section $A = 33 \times 10^{-1}$ cm² and the length $l = 5{\cdot}15 \times 10^{-8}$ cm, we find $E = 89 \times 10^{1}$ dyn/cm².

To compare experimental values of Young's modulus with this theoretical value, they must have been measured at a high frequency on material that is highly crystalline, highly orientated and dry. Native cellulose fibres that are already highly crystalline are flax, hemp, jute, and ramie. The highest Young's moduli experimentally observed are given by such fibres that have been wetted and dried under tension to increase further the already high degree of orientation. Thus, at a frequency of about 7 kc/s, ramie that has been wetted and dried under tension has a dynamic Young's modulus of 90×10^{10} dyn/cm² and linen treated the same way has a dynamic Young's modulus of $78\text{–}108 \times 10^{10}$ dyn/cm². These values are quite close to the theoretical value of 89×10^{10} dyn/cm².

STRESS–STRAIN CURVES

Figure 7 shows typical stress–strain curves for a number of cellulose fibres. The native cellulose fibres, cotton and flax, follow the general rule of increase in modulus and strength and decrease in breaking extension with increase in molecular orientation; the same effect is clearly shown with viscose rayon as we pass from the ordinary low-tenacity viscose rayon as used for apparel purposes through the medium-tenacity types, such as Tenasco used in tyre cords, to the high-tenacity types such as Durafil and Fortisan. The strongest viscose rayons have strengths as high as 110×10^{8} dyn/cm² (8 g/denier).[24] Acetate fibres show a similar increase in strength and decrease in breaking extension with increase in molecular orientation produced by stretching the filaments during manufacture.[25]

The new triacetate fibres such as Arnel and Tricel have stress-strain curves very much the same as that of ordinary secondary cellulose acetate at normal room temperatures. The advantages of triacetate include its higher melting point (290°–300°, compared with 235° for ordinary acetate, around 250° for Terylene and around 260° for nylon) which gives safer ironing and enables the thermoplastic properties of the material to be utilized in producing durable pleats in fabrics. The fully substituted cellulose also has a lower moisture regain of 4·5 per cent (after heat-setting, about 3 per cent) compared with 6·5 per cent for ordinary acetate. The lower water imbibitions of 10 per cent for heat-set triacetate and 16 per cent for normal triacetate compared with 26 per cent for ordinary acetate lead to advantages in quick drying. This modification in the properties of acetate fibres reflects the modern trend towards producing cellulose fibres with some of the advantageous properties of the synthetic fibres without losing desirable mechanical properties and, if possible, improving the mechanical properties. For example, triacetate fibres have a higher ratio of wet strength to dry strength than ordinary secondary acetate.

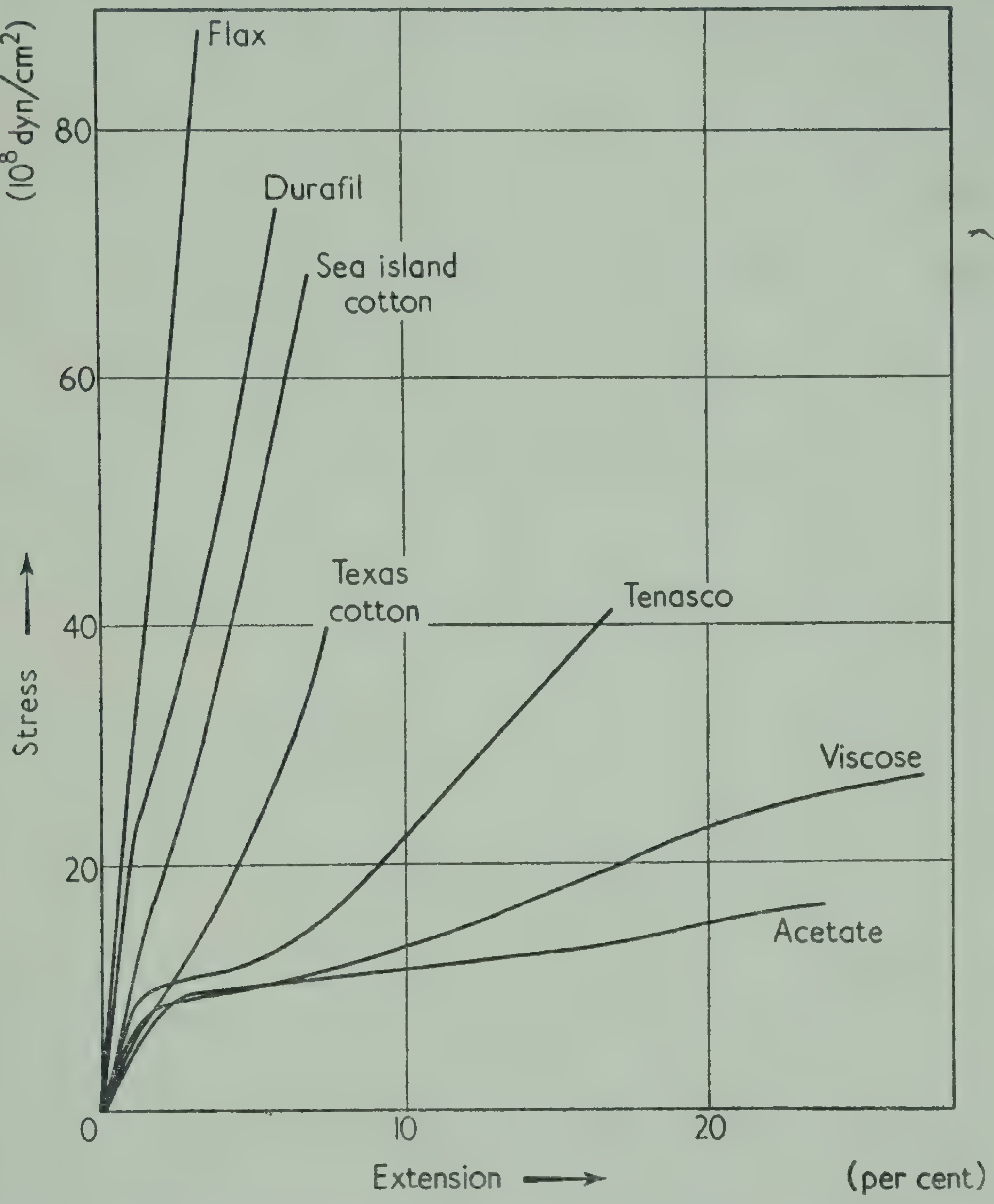


Figure 7. Stress–strain curves for cellulose fibres at 65 per cent R.H. and 20°

The influence of humidity on the stress–strain curves of a number of cellulose fibres is shown in Figure 8. The native cellulose fibres increase in strength with humidity, owing to the plasticizing action of the absorbed water producing a reduction in internal strain and a more uniform distribution of internal stress. Regenerated cellulos fibres lose strength with increasing moisture content owing t reduced cohesion between the chain molecules. Treatment of thes fibres with urea-formaldehyde reduces swelling by introducin covalent cross-linkages and blocking access to hydroxyl groups thereby increasing wet-strength and elastic recovery but tending t produce embrittlement of the fibre unless the process is carefull controlled.

The reversible effect of temperature on mechanical propertie

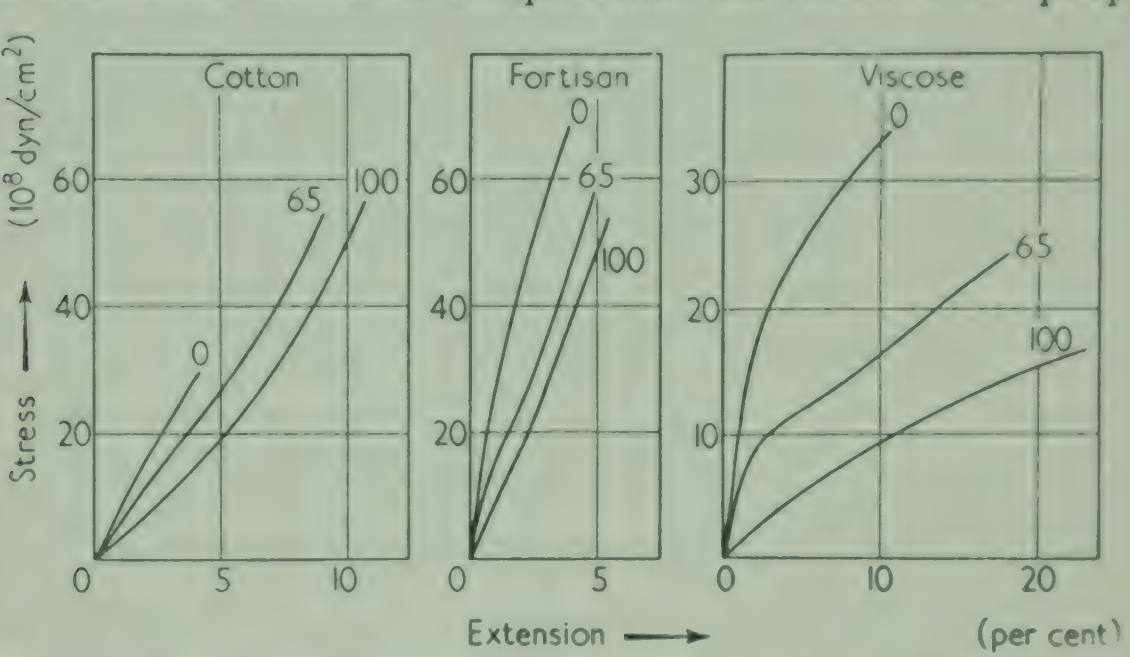


Figure 8. The effect of humidity on the stress–strain curves of cotton, Fortisan, and viscose rayon. Numbers on curves give percentage relative humidity

is more marked for cellulose acetate than for other cellulose fibres, as may be expected from its molecular structure. For example, the dependence of the wet strength and breaking extension of viscose and acetate is shown in Table 3 from which it will be seen that at 100° the acetate fibre is much the weaker and more extensible.

The strength of viscose rayon shows very little change up to 60°, an observation confirmed by the work of Clark[26] et al., but this is followed by a more rapid decrease as 100° is approached. The rate of relaxation of stress of viscose rayon extended 4 per cent in water is also observed to increase appreciably above 60°. This is explained on the assumption that above 50° there is a rapid rise in the rate of dissociation of intermolecular bonds. This explanation also fits the fact that the swelling of viscose rayon is a minimum[27] at about 55°.

Although the data given in Table 3 show a slight increase with temperature in the breaking extension of wet viscose rayon, Clark et al.[26] give results for their sample which show a maximum breaking extension between 50° and 60°. A similar maximum is exhibited by cotton[28] immersed in water at 30° and this can be explained on the same hypothesis of competing rate processes leading to a sharp rise in the rate of dissociation of hydrogen bonds at about 30°.

Moisture content and temperature control the mechanical properties of cellulose by virtue of their effect on the bonding between cellulose chains in the non-crystalline regions, so that cellulose changes from an elastic glass in the dry state to a partially rubber-like material in the wet state at high temperatures.

Table 3: Effect of temperature on wet strength and breaking extension

Temperature	Strength as percentage of that at 20°		Breaking extension as percentage of that at 20°	
	Viscose	Acetate	Viscose	Acetate
20°	100	100	100	100
60°	96	75	101	124
100°	85	40	106	(190)

The equation of state for a material under tensile force, f, at constant pressure and temperature, is given by the equation (see ref. 2, p. 1300)

$$f = \left(\frac{\partial U}{\partial l}\right)_T - T\left(\frac{\partial S}{\partial l}\right)_T$$

where U = internal energy, l = length, T = absolute temperature and S = entropy.

This equation states that for a small reversible change in length, the tension in the fibre is the result of a change in potential energy, e.g. straining of bond angles or bond distances, and of a change in the entropy or statistical configuration of the chain molecules. If the entropy increases on stretching, transition from a more-ordered to a less-ordered state is indicated. When the entropy decreases during stretching, i.e. when $(\partial S/\partial l)$ is negative, the molecular chains become more ordered.

It can be shown by classical thermodynamics that

$$\left(\frac{\partial S}{\partial l}\right)_T = -\left(\frac{\partial f}{\partial T}\right)_l$$

and from the property of differentials

$$\left(\frac{\partial f}{\partial T}\right)_l = -l\left(\frac{\partial f}{\partial l}\right)_T \cdot \frac{1}{l}\left(\frac{\partial l}{\partial T}\right)_f$$

so that the change of entropy on stretching can be obtained from either (i) the change of tension with temperature at constant length, or (ii) the product of the modulus at constant temperature multiplied by the coefficient of linear expansion at constant tension. All this theory is subject to the changes being reversible.

The data in Figure 9 for wet and dry viscose rayon[29] show that in these experiments this condition was satisfied, for the dots represent values of the moduli calculated from reversible changes of tension and length with temperature and fall close to the observed curves.

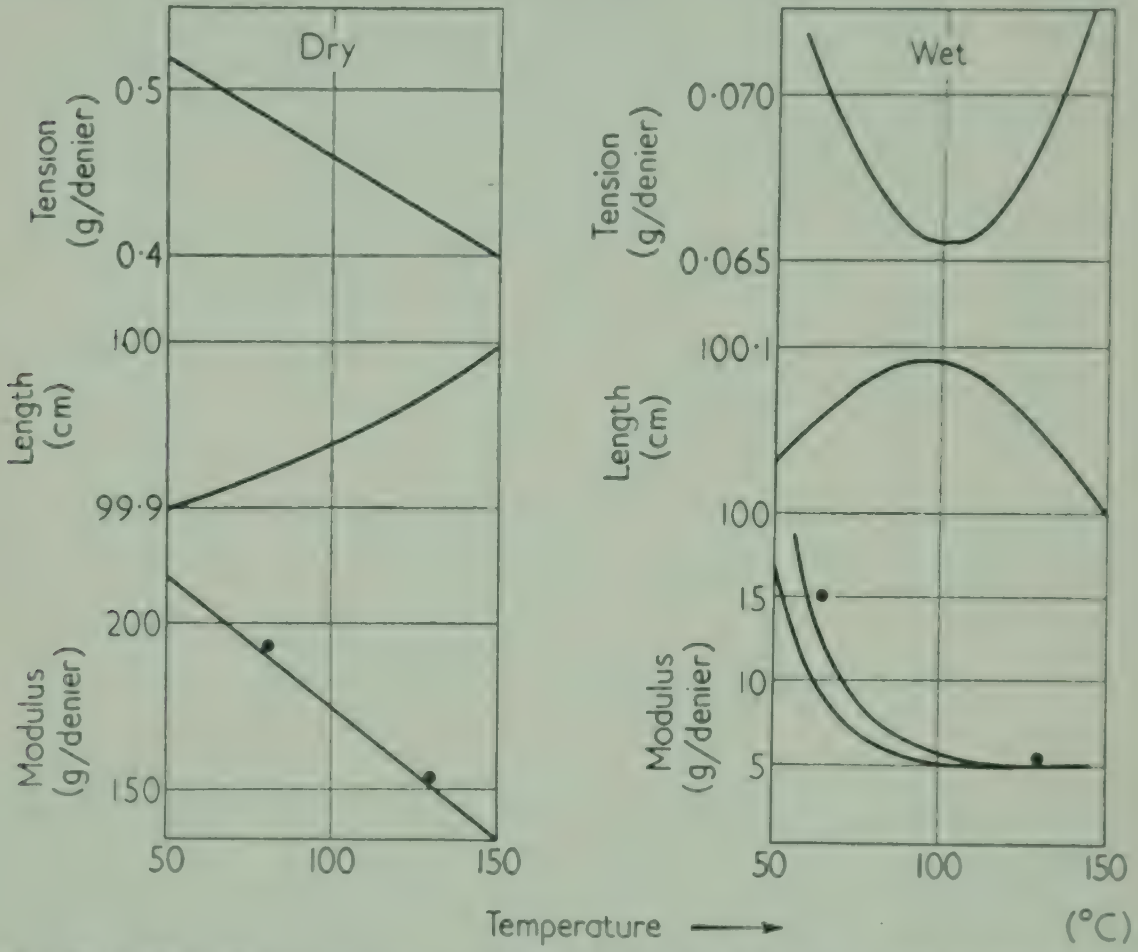


Figure 9. The effect of temperature on the tension at fixed length, length at constant tension and modulus of deformation of viscose rayon in the bone-dry and wet states. The dots represent calculated values

The conclusions that can be drawn from these curves are that for dry viscose rayon, the deformation involves almost entirely elastic deformations of valence bonds and angles, whereas for wet viscose rayon, at high temperatures, the molecular segments are solvated in water and have rubber-like properties. At 50°, cross-links form, and below 50°, the life and number of cross-links increases and the nature of the deformation changes from one controlled by entropy changes to one controlled by internal energy changes.

It has already been seen that the time factor plays an important part in the mechanical behaviour of cellulosic materials, as it does in fact with all fibrous materials based as they are on linear macromolecules held together by weak forces, which can be broken by thermal agitation. Figure 10 shows typical curves for viscose rayon obtained at different rates of extension, the time to break ranging from 0·025 sec to 5 hr. The initial modulus, yield stress and tensile strength increase linearly with log(rate of extension). This is a fairly general rule which is followed by other fibrous materials.[30] When the rate of extension becomes sufficiently high, the stress is propagated as a shock wave[31] and there is a critical velocity which is a characteristic property of the material and is given by the equation

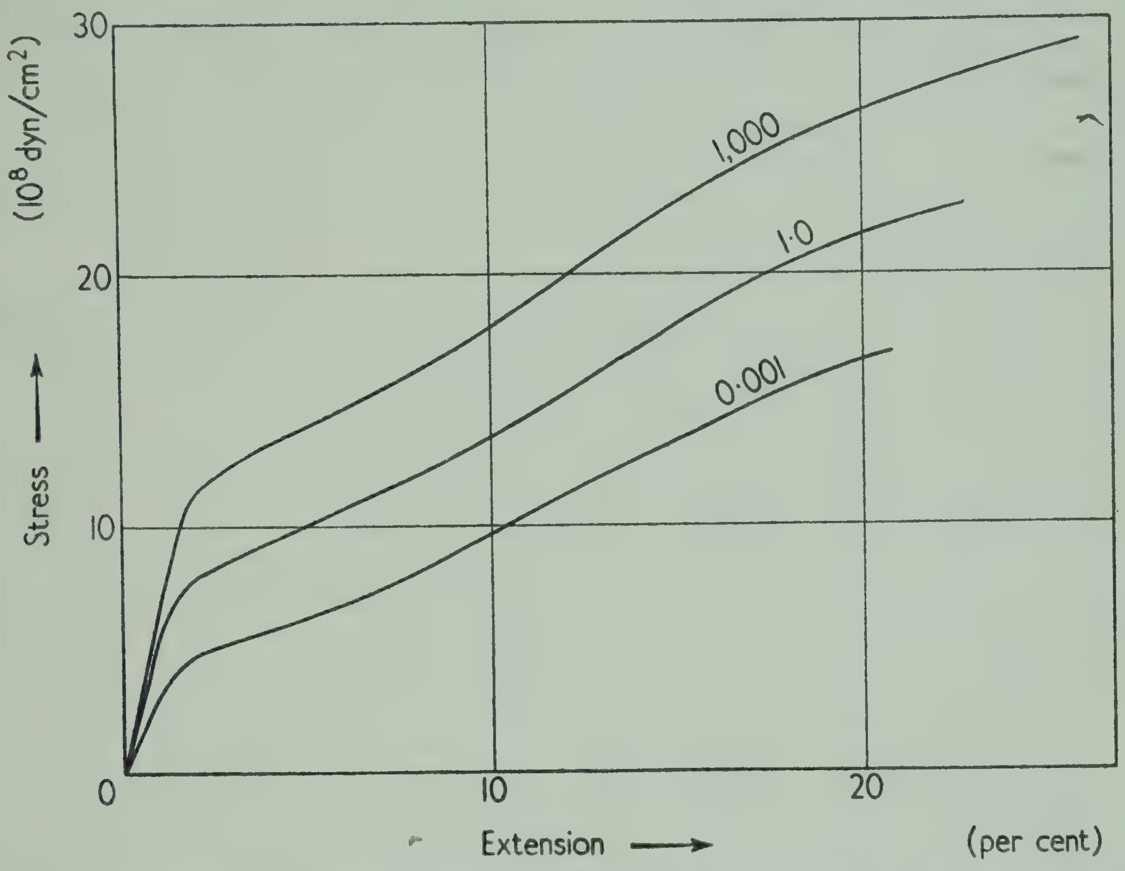


Figure 10. The effect of rate of extension on the stress–strain curve of viscose rayon at 65 per cent R.H. and 20°. The numbers on the curves give the rates of extension in per cent/sec

$$V_c = \int_0^{e_r} \sqrt{\left[(1/\rho).(\mathrm{d}f/\mathrm{d}e)\right]}.\mathrm{d}e$$

where ρ = density, f = stress and e_r = breaking strain. When Hooke's law is obeyed, this equation reduces to

$$V_c = e_r\sqrt{\frac{E}{\rho}}$$

where E is the dynamic Young's modulus. $\sqrt{(E/\rho)}$ is the velocity of sound in the material. Critical velocities for cotton and nylon, extrapolated from experimental data,[32] are 130 and 228 m/sec. For Fortisan, the critical velocity can be calculated to be about 270 m/sec.

THEORETICAL TENSILE STRENGTH

By comparing the observed tensile strength with that calculat theoretically, it should be possible to throw some light on the mec anism of rupture, i.e. to say whether breaking takes place by chai breakage or chain slippage.

An estimate of the maximum observable tensile strength cellulose can be obtained in the following way. A mean strength more than 200 × 10^8 dyn/cm^2 has been observed directly for fla fibres broken at 65 per cent R.H. in 20 sec with test length 0·2 mm Consider a flax fibre broken in 0·01 sec, in water, with test lengt of 0·2 mm. Such a fibre would have a breaking stress of at leas 290 × 10^8 dyn/cm^2.

An upper limit to the theoretical tensile strength of cellulos which could never be realized in practice, has been calculated b Mark[33] from known molecular data for a perfectly orientated perfectly crystalline cellulose fibre by assuming that all the chai molecules rupture simultaneously. The cross-sectional area of chain molecule is 33 × 10^{-16} cm^2 and hence the number of molecule passing perpendicularly through 1 cm^2 of cross-section is 3 × 10^{14} The force necessary to rupture one cellulose chain is calculated b assuming that the chain breaks at the –C—O—C– links whic connect adjacent glucose residues. Now the maximum forc required to separate the two carbon atoms in an aliphatic C— bond[34] is 5·6 × 10^{-4} dyn and if we assume that the force necessar to separate the carbon and oxygen atoms in the C—O bond is o the same order of magnitude, we arrive at a breaking stress o about 1,680 × 10^8 dyn/cm^2 for the maximum strength of crystallin cellulose. This value is much higher than any observed value, a would be expected, since highly crystalline fibres such as flax contai only about 60–70 per cent crystalline cellulose and have man imperfections of structure that always act to reduce the strength.

Since the observed strength of cellulose fibres falls short of th calculated maximum strength it has sometimes been suggested tha the chain molecules slide over each other rather than break. Ther are many facts to indicate that this is not so in the case of nativ cellulose, at least. Thus, the strength increases with increase i moisture regain, the stress–strain curve becomes concave to th stress axis near rupture and shows no flow, the breaking load o ramie fibres does not change noticeably after careful nitration an acetylation in the fibrous form. All these facts suggest that chai breaking is primarily involved in the case of native fibres.

In a recent paper[35] on the effect of temperature on the tensil strength (f) of cotton and highly orientated viscose rayon, it has been observed that $f = A + B/T$, where A and B are constants, and attempts have been made to explain this in terms of reaction-rate theory applied to the slipping of chain molecules over each other. The explanation has not succeeded because the fundamental concept of rupture purely by chain slippage does not hold for highly orientated materials.

In the case of regenerated cellulose fibres in which the degree of polymerization is lower, it is reasonable to suppose that the cellulose chains do slide over each other and the number of chain molecules actually ruptured in breaking a fibre will be fewer than in native cellulose fibres. For an average degree of polymerization of 300, de Boer[34] calculated a maximum tensile strength of 120 × 10^8 dyn/cm^2, when the chain molecules slide over each other. The longer the chains, the less likely are they to slip. From considerations of the influence of test conditions on the strength of fibres, it seems likely that the strength of highly orientated viscose rayon, which is 110 × 10^8 dyn/cm^2 (8 g/denier) under normal test conditions, could easily be as high as 250 × 10^8 dyn/cm^2 when very short lengths of the dry fibres are broken in short times. Since the degree of crystallinity of such rayons is only 30–40 per cent much of the fibre material will be an imperfectly arranged state so that rupture will start at the weakest spot in a cross-section and follow a course depending on the distribution of internal strain, and the observed strength would be expected to be only a fraction of the theoretical 120 × 10^8 dyn/cm^2. Under these conditions it seems likely that considerable chain breakage must occur.

In the case of ordinary viscose and acetate, the relatively low strength and plastic flow shown in their stress–strain curves indicate that the fibres rupture mainly by chain molecules tearing apart. This is especially the case with wet rayon fibres.

The strength of cellulose has been dealt with at some length since a clear idea of the factors controlling strength should allow one to understand what might happen in any chemical modification designed to improve certain properties such as resistance to heat and rotting, or reduction of moisture absorption, whilst at the same time preserving the valuable tensile properties of the modified cellulose.

CHEMICALLY MODIFIED CELLULOSE

It is proposed in this section to consider the effect of a number of chemical treatments on the mechanical properties of cellulose fibres. Such treatments, made to improve certain physical or chemical properties, are, for example, mercerization, acetylation cyano-ethylation, and decrystallization.

Mercerization of cotton with caustic soda is a well-known proces which is used mainly to give higher lustre and greater dye affinity Depending on the conditions used, the mechanical properties are changed, usually to advantage. For example, mercerization with tension applied to the yarn gives high lustre, improved strength and reduced breaking extension leading to greater dimensional stability in spite of the increase in moisture regain. On the other hand, more recent developments[36,37] in which the cotton fabric is allowed to shrink freely in caustic soda solution of high concentration, have produced a fibre with increased extensibility and therefore useful for bandages and flexible plastic-coated or laminated fabrics.

Cotton fibres do not have a high breaking extension since they are highly crystalline and have a good degree of molecular orientation. For some purposes, a greater extensibility is desired and another way of attaining this end, other than by mercerization in a slack state, is to reduce the degree of crystallinity by treatment with anhydrous liquid ethylamine and removal of the amine with ice-cold water.[38] This process reduces the crystallinity by a large amount and gives the fibre an increased extensibility, and increased work of rupture, without lowering its strength. From a given strain, the elastic recovery is higher after decrystallization.[39] The moisture regain and chemical reactivity of the fibre are increased by this process.

Cyano-ethylation of cotton has become a commercial process since supplies of acrylonitrile have become easily available and cheaper (see Chapter 7). The influence of the degree of cyano-ethylation, measured by the nitrogen content, on a number of mechanical properties is shown in Figure 11. Yarn and fabric strength begin to decrease appreciably when the nitrogen-content exceeds about 5 per cent and 7 per cent, respectively. Yarn and fabric breaking extension increase. Flex-abrasion resistance falls to a minimum at about 6 per cent nitrogen-content and then increases, whereas flat-abrasion resistance increases markedly. The right-hand ordinate scale applies only to the flat-abrasion resistance curve.

Acetylation of cotton cellulose with retention of its fibrous form has been practised for many years with the object of reducing moisture regain, increasing the resistance to heat tendering, increasing the electrical resistance and modifying the dyeing properties. More recently interest has been revived[40–42] and the properties of partially and fully acetylated cottons have been investigated in some detail.

Although rot-resistance is the main advantage derived from partial acetylation, heat resistance is improved, e.g. high temperatures can be withstood for about ten times as long for a given loss in strength.[43] The improvement of heat resistance with increasing degree of acetylation is shown by the curve in Figure 12 marked ' strength retained '; the period of heating was 4 days at 160°. The breaking load of air-dry yarn is little affected by acetylation up to an acetyl content of 25 per cent but the bone-dry yarn breaking load increases, and the wet yarn breaking load decreases, with increasing acetylation, as shown in Figure 12.

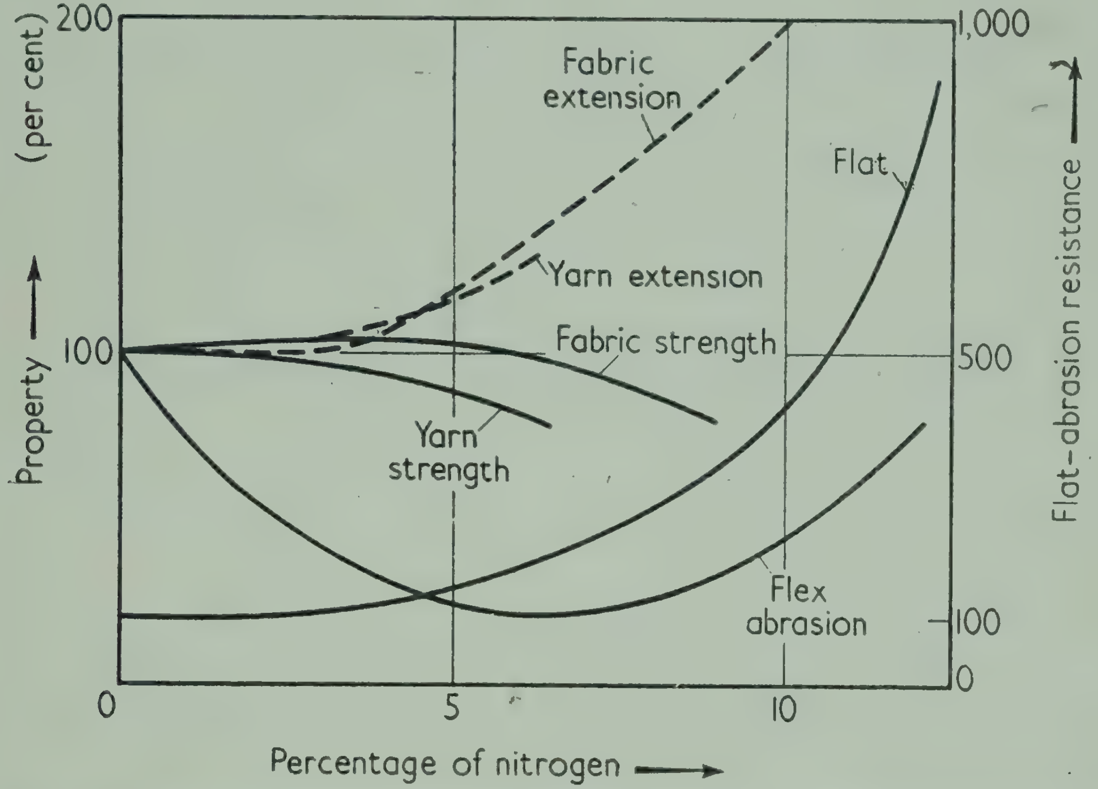


Figure 11. The change in mechanical properties of cotton with degree of cyano-ethylation[53]

Fully acetylated cotton is claimed[41] to have many properties superior to other cellulose fibres, including triacetate fibre. It has good wet and dry tenacity and flex-abrasion endurance, together with some thermoplasticity, but is safe to ironing temperatures up to 250°. Naturally, the fully substituted cellulose has a low moisture regain (2 per cent at 65 per cent R.H.). Some idea of the versatile nature of this material can be judged from the data in Table 4, showing the effect of heat and stress on the strength, elongation, growth and elastic modulus of fully acetylated cotton fabric.

The properties do not change appreciably under conditions of very little tension which are used for the heat-setting of solvent-spun triacetate. However, a moderate tension applied for a short time whilst heated causes an appreciable increase in strength and modulus and greatly reduces the growth on cyclic loading, s enhancing the dimensional stability of the fabric which is alread good on account of the low moisture regain. Such treatment will of course, reduce the draping quality of a fabric. The flat-abrasio endurance is increased in the fully acetylated cotton but the flex-abrasion is reduced. This is possibly due to the increase in extensibility and coarseness of the fibres and yarns on acetylation. Increased extensibility and coarseness of cotton fibres produces an increase in flat-abrasion resistance. Increased coarseness produces greater bending strains on flexing and so lowers flexing endurance.

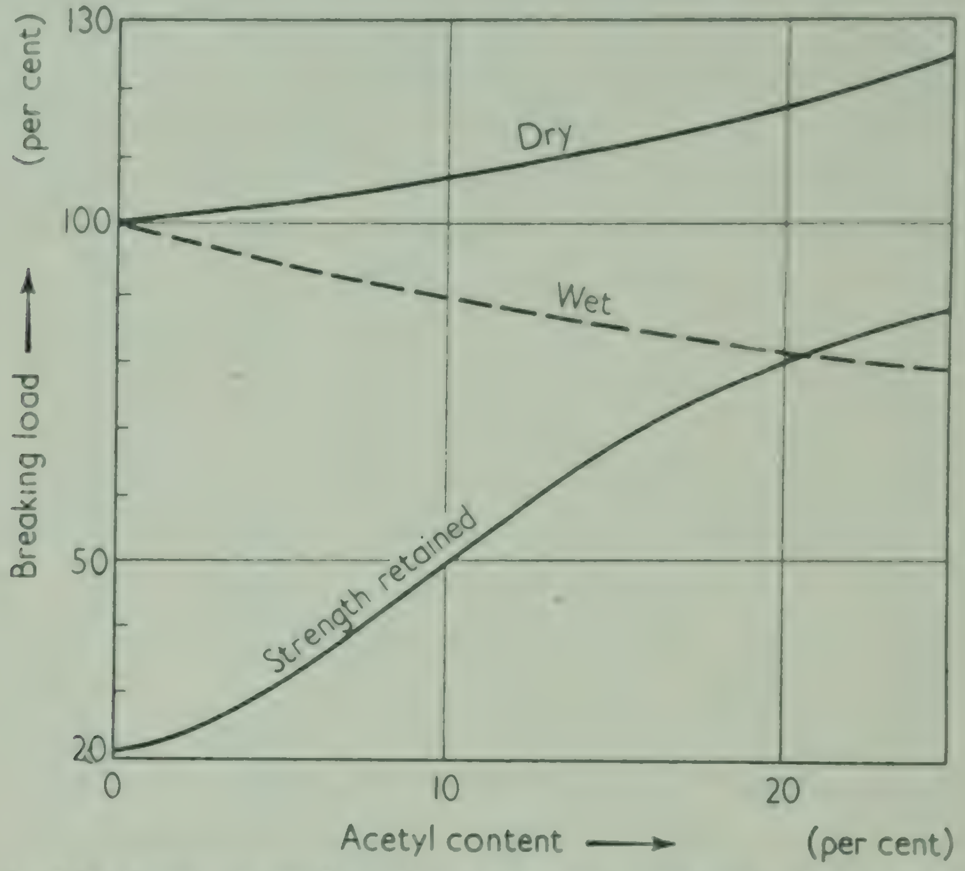


Figure 12. The change in tensile properties of cotton yarn with degree of acetylation[42]

Table 4: Effect of heat and stress on fully acetylated cotton fabric[36]

Treatment	Breaking load (lb)	Breaking extension (per cent)	Growth* (per cent)	Relative modulus (per cent)
Scoured	108	7·1	1·9	—
Fully acetylated	152	13·1	5·1	100
Same, 1 min at 200°, no load ...	152	12·9	5·0	76
Same, 34 lb load 1 min, no heat	154	11·8	3·7	85
Same, 1 min at 200°, 34 lb load	173	3·9	0·3	212

* Growth refers to the displacement of the tenth-cycle hysteresis loop along th extension axis, the upper limit of the loop being one-third of the strength.

INFLUENCE OF RADIATION

There has been a suggestion that exposure of polymers to γ-rays or neutrons may induce cross-linking and improve their strength properties. The investigations that have been made[44–46] show that, as far as cellulose fibres are concerned, the mechanical properties are worsened in general, except for secondary cellulose acetate exposed to intermediate doses of γ-rays in vacuum which showed an increase in breaking strength and extension. Oxygen is a strong contributor to degradation in the presence of radiation and elimination of oxygen results in a marked decrease in the strength loss shown by nylon 66 but not in the strength of cellulose fibres. Dacron and Orlon appear to be the least affected by γ-radiation.

Under neutron bombardment in an atomic pile for 26 hr with a flux of $2 \cdot 3 \times 10^{17}$ neutrons/cm^2, Orlon lost about one-quarter of its strength, nylon lost more than one-half, and cellulose fibres lost nearly all their strength. The general conclusion is that γ-radiation and neutron bombardment have no beneficial effect and, when severe enough, lead to considerable degradation.

CONCLUSION

Research continues with a view to filling many gaps in our knowledge. For example, recently,[47] the creep of highly nitrated cellulose over a wide time range has been shown to obey the equation $e = e_0 + \beta t^{1/3}$ where e is the strain, t is the time and β a parameter depending on the stress. This equation was first used by Andrade[48] to describe the creep of metals and a similar equation involving $t^{1/3}$ was used by Peirce[49] to describe the relaxation of torque in fibres held at constant twist.

Some light is shed on the relation between structure and mechanical properties by studies of the effect of molecular weight distribution on the creep of cellulose acetate,[50] by measuring the temperature dependence of rigidity modulus and internal friction of cellulose acetate,[51] and by studies of the dependence of rigidity on moisture absorption[52] which have shown that the behaviour of different types of native and regenerated cellulose fibres can be described by a single equation.

Further research on the mechanical behaviour of cellulose in its different forms, covering a wide range of humidity, temperature, and time and using modern experimental techniques, will ultimately yield the common factors which determine the dependence of mechanical properties on fibre structure.

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9

SORPTION OF WATER BY CELLULOSE AND STARCH

THE sorption of water by cellulose and starch is important from two distinct points of view. These substances are able to take up large amounts of water from the surrounding atmosphere while remaining apparently dry, and hence it is necessary for those concerned with processing to know how the amount of water taken up depends on the variables that control it. Information of this kind is of intrinsic value as data, if only for the adequate control of testing procedures and commercial transactions. But this aspect of the subject will only occasionally be mentioned; instead attention will be directed mainly to the underlying scientific basis of the phenomena, and in particular to the information it provides about the fine structure of these materials.

CELLULOSE

Cause of Sorption

Amongst much that is vague and uncertain, the principal underlying cause of the sorption is not seriously in doubt. The reduction in hygroscopicity that accompanies the formation of derivatives by combination to the hydroxyl groups,[1,2] together with studies of the primary sorption at low relative vapour pressures[3,4,5,6] and of the energetics of the process,[7] make it clear that the sorption is attributable to hydrogen-bonding of water molecules to the available hydroxyl groups of the substrate, i.e. those in the amorphous regions and on the surfaces of the crystallites. Measurements of the energy changes accompanying sorption suggest that the initial strong sorption occurring at the lowest values of the relative vapour pressure results from the linking of one molecule of water to a pair of suitably placed hydroxyl groups of the cellulose, while at later stages combination to one hydroxyl group and to the oxygen atoms will occur, while finally water molecules will be able to condense only on already heavily covered surfaces, and the thermodynamics of the process will approach those of condensation in bulk. Further evidence in favour of this view is to be found in the fact that although, as will be seen later, very large differences of hygroscopicity are observed between different kinds of cellulose, there is no difference in the energy changes accompanying the primary sorption,[7] indicating that the differences in the amounts taken up are attributable to differences in the number of hydroxyl groups available, and not to any difference in intensity of sorption at any one group.

The Sorption Isotherm

Let us consider first the sorption isotherm—the relation at a fixed temperature between the amount of water taken up and the relative vapour pressure (or 100 times that value, per cent relative humidity) of the atmosphere to which it is exposed. The relation between these two quantities is illustrated diagrammatically in curve (a) of Figure 1; the curve is sigmoidal in shape, and several attempts

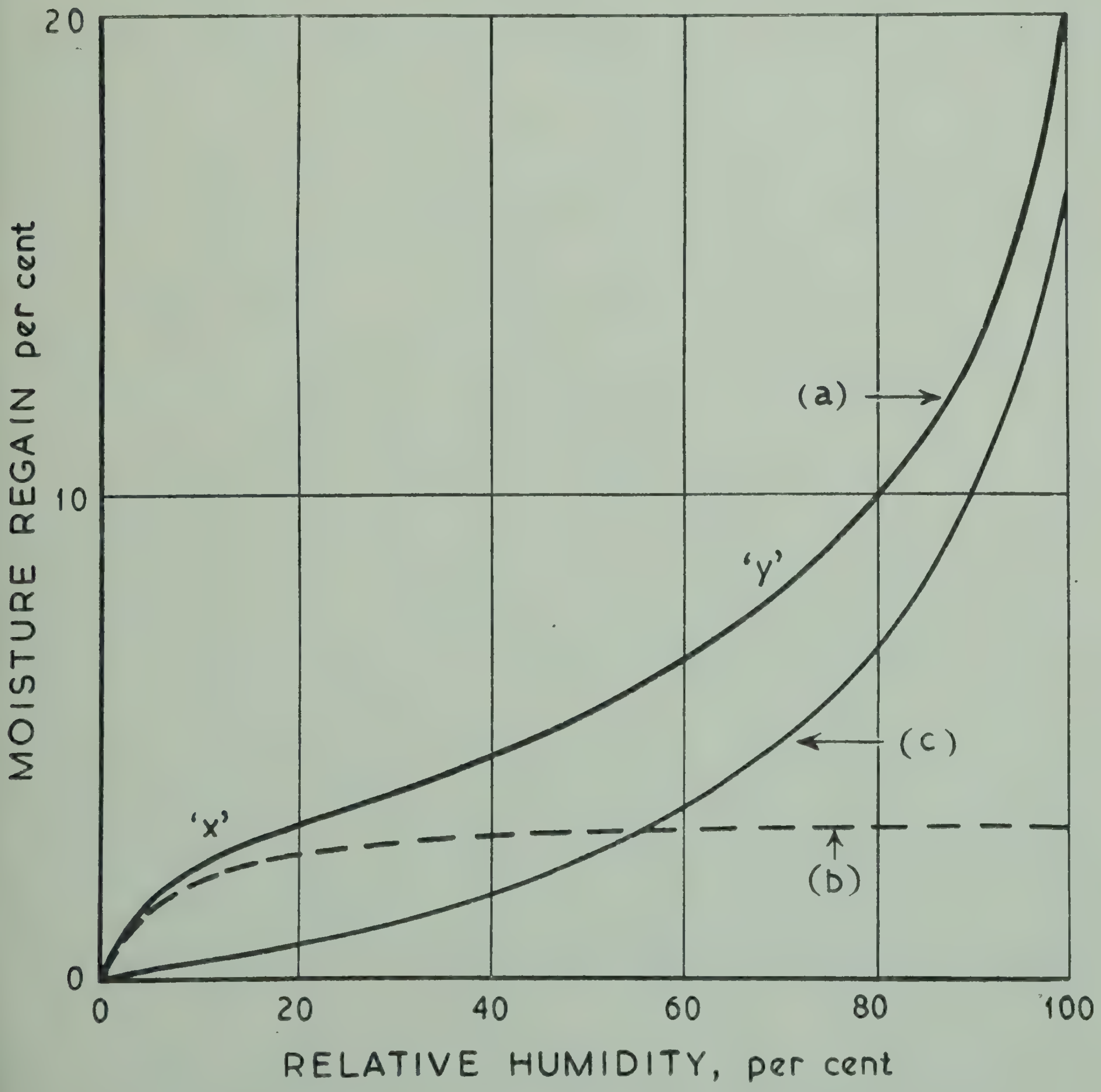

Figure 1. Analysis of sorption isotherm

have been made to provide a theoretical explanation of that shap The best known is probably that of Peirce,[8] which presupposes tha the water is held in two phases, one in which one hydroxyl group i each anhydroglucose residue can take up one water molecule, an the other in which the water molecules fill the available spaces unde forces similar to those in a liquid. According to this theory, th relation between relative vapour pressure and the amount take up in the first phase is indicated by the curve (b) of Figure 1, an that in the second phase by curve (c), the sum of the two giving th sigmoidal curve (a). This theory has recently been modified b Meredith,[9] who more plausibly removes Peirce's restriction of on water molecule per hexose unit, but restricts sorption to th *available* hydroxyl groups in the amorphous regions and on th crystallite surfaces. Hailwood and Horrobin[10] applied a differen argument to achieve a result similar to that of Peirce; according t their theory, curve (b) results from combination to form a hydrat with the cellulose, while (c) results from water existing in solution the sum of the two again yielding the experimental curve (a) Purves[11] and his collaborators have applied their elegant thallatio technique to studying the relative reactivities of the differen hydroxyl groups in the glucose residue, and have found that th primary hydroxyl is appreciably more reactive than the two secondaries. They[12] have suggested therefore that the initial sorptio up to the point x on curve (a) is ascribable to bonding of wate molecules to the primary hydroxyl groups, and that the sorptio between the points x and y is occurring on the two secondar hydroxyls, the remainder of the curve representing some form o looser binding. As supporting evidence, they point out that th total sorption between the points x and y is approximately twice tha up to the point x. On the other hand, the difference of reactivit between primary and secondary hydroxyl groups is not big enoug to justify the assumption that sorption on the primary hydroxyl is virtually complete before that on the secondaries begins; moreover, the sigmoidal curve is a very smooth one, and the selection of th points x and y arbitrary, so that it would not be difficult to adjus their selection so as to satisfy any quantitative requirement withi reason. This general question of the theoretical interpretation o the sorption isotherm must, therefore, be left open at present fo among several theories, little is surely known.

Sorption Hysteresis

So far the sorption isotherm has been discussed as if there were unique relation between the amount sorbed and the relative vapou pressure; actually there is not, as the experimental isotherm shown in Figure 2 demonstrates.[13] This is the isotherm for a well-scoured cotton, which may be taken as pure cellulose; as cotton has been studied in greater detail than any other form of cellulose much of the subsequent discussion will deal with it, though most of the general conclusions will be applicable to all forms of cellulose. The lower of the two curves was obtained by adding water to dry cotton, and the upper by removing it from cotton that had been allowed to come to equilibrium with a saturated atmosphere. The two curves are quite distinct, and the two values corresponding to any given vapour pressure do not approach one another more closely if the samples are left in the same atmosphere for a long time; they represent true, though different, equilibria. This phenomenon has come to be known as sorption hysteresis,[14] and the curve obtained by adding water to the dry material is usually called the absorption curve, and that obtained by removing water from the saturated material, the desorption curve.

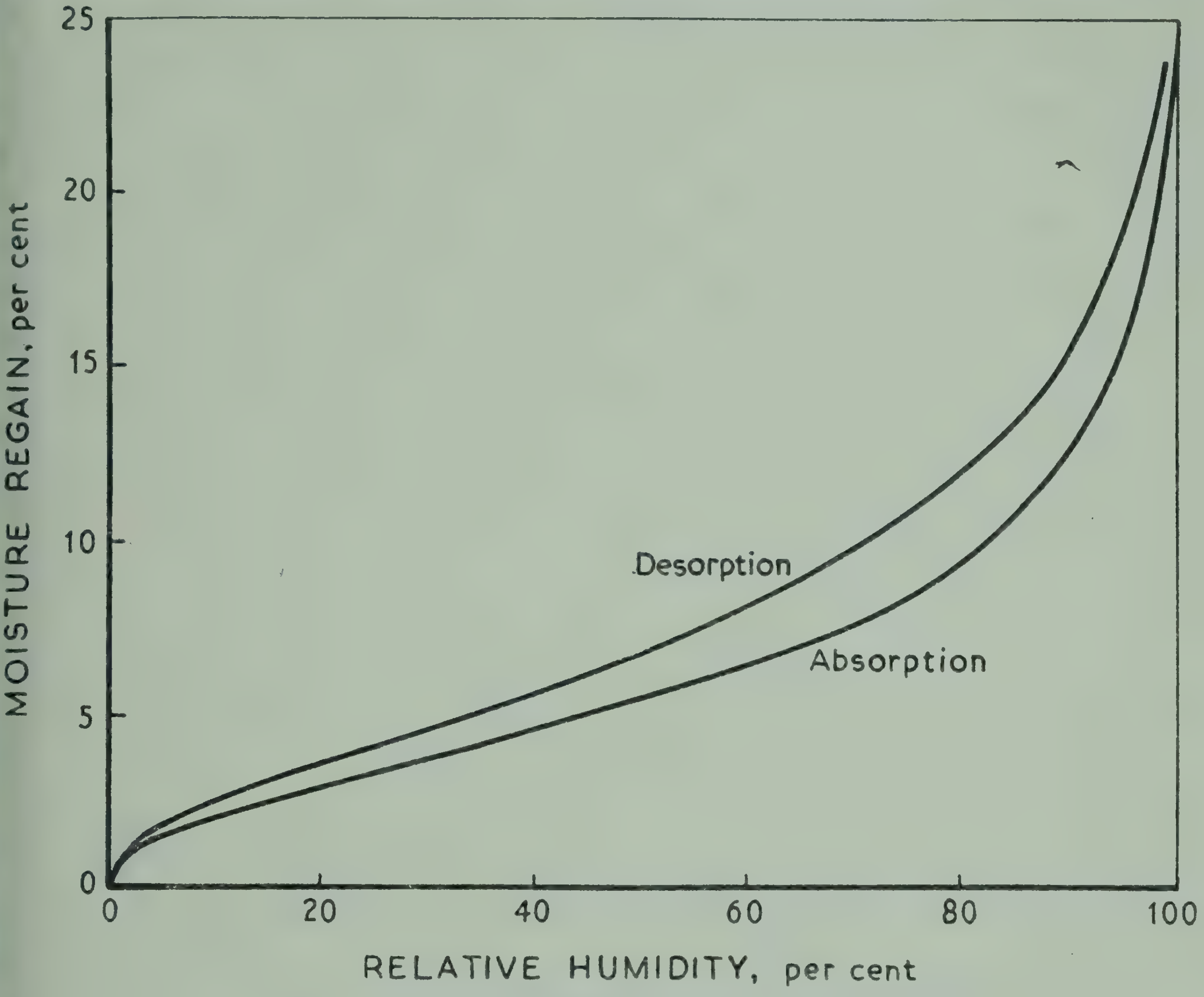

Figure 2. Sorption of water by scoured cotton at 25°

In the earlier years of our knowledge of hysteresis it was suggested[15] that the phenomenon was not a real one but an artifact, attributable to inadequate removal of air from the experimental system. It is not disputed that another, quite different, kind of hysteresis can be caused in this way, but the suggestion that this explanation is applicable to the kind of sorption hysteresis shown by cellulose, starch, etc., is now completely discredited.[16] There is, however, one difficulty that still has to be resolved. Within recent years, several workers[4,17] have reported that the initially determined absorption and desorption curves were not reproducible, and that the material under investigation had to be taken through several cycles from dryness to wetness and back again before it settled down to give completely reproducible absorption and desorption curves; this initial cycling they have termed stabilization. In a very recent paper,[18] for example, the curves of Figure 2 are referred to as those of ' unstabilized ' cotton. This is, of course, correct in that the cotton was not submitted to a stabilization process of the kind indicated; it was nevertheless completely stable, in that it was possible to take it through several completely reproducible cycles. The stability of ' unstabilized ' cellulose has also been demonstrated by other workers,[19] yet there is no reason to doubt the findings of those who found a stabilization process necessary. The cause of this discrepancy remains unknown; it is most probably related to the method of experimentation or of preparation of the sample.

Returning now to the phenomenon in general, we find that the amount of water taken up is not determined uniquely by the vapour pressure and the temperature, but depends also on the previous history of the sample. Moreover, it has been shown that these two curves are to be taken not as the locus of all possible equilibria, but as defining an equilibrium area, any point in which can represent the amount of contained water under suitable conditions of relative vapour pressure and prehistory. This is illustrated by the curves[20] of Figure 3, from which it is clear that more limited cycles not extending to either the dry or the saturated condition do in fact follow intermediate paths.

Since the sorption isotherm is an area, it is important to have some knowledge of the curves that define that area. There is little difficulty about the lower limit; the absorption curve from dryness is the locus of minimum moisture contents for any given material. The exact definition of the upper limit is less simple. The curves of Figure 4 show that the exact configuration of the desorption curve above a relative humidity of about 80 per cent depends on the amount of water added to the sample in excess of that required to saturate it, the limiting curve being obtained by removing water from completely wet cotton.[20] For the particular sample used to obtain Figure 4, the absorption curve reaches the saturation vapour pressure at about 23 per cent added water, but the desorption curve determined from wet cotton leaves it at about 40 per cent added water. Moreover, it has been found that the rate of drying of wet cotton is constant until the moisture regain falls to about 40 per cent, after which a sharp decrease in the rate occurs. The most reasonable explanation of this high-humidity indeterminacy of the desorption curve is that if water in excess of the amount required for saturation is added to cotton in bulk, the spaces between the fibres will become filled, and their subsequent emptying will cause a reduction of the vapour pressure of their contained water, due to the curvature of the water surfaces in these spaces, even while the total amount of water present is still in excess of the saturation value. This

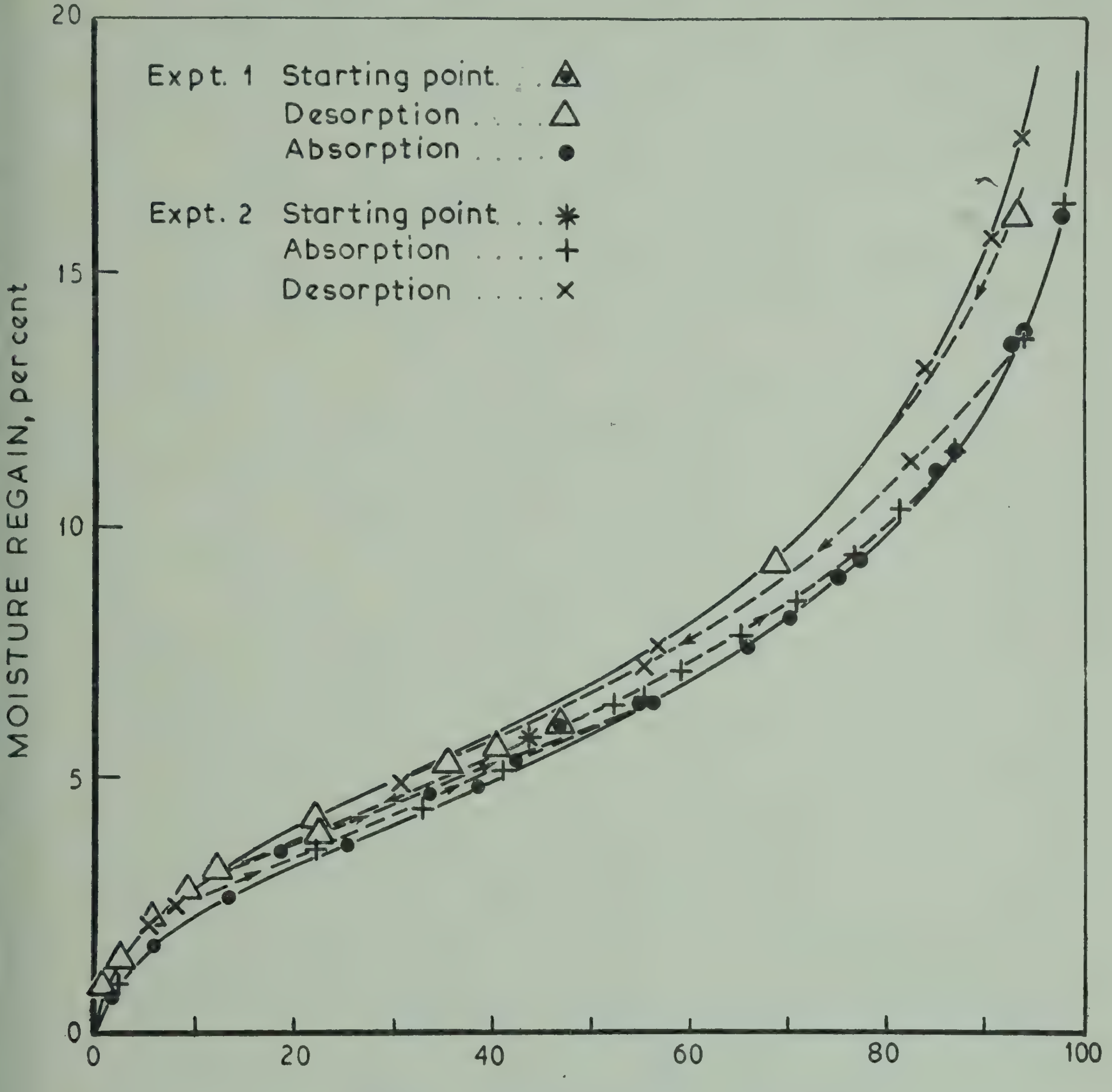

Figure 3. Sorption of water by raw cotton at 25°

explanation, therefore, attributes the variability of the hygroscopicit at high humidities, not to an intrinsic property of the material, bu to its state of aggregation, and it is supported by the fact that it i non-existent in the isotherm for cellulose film, the absorption an desorption curves of which have a common meeting point at 10 per cent relative humidity.[1]

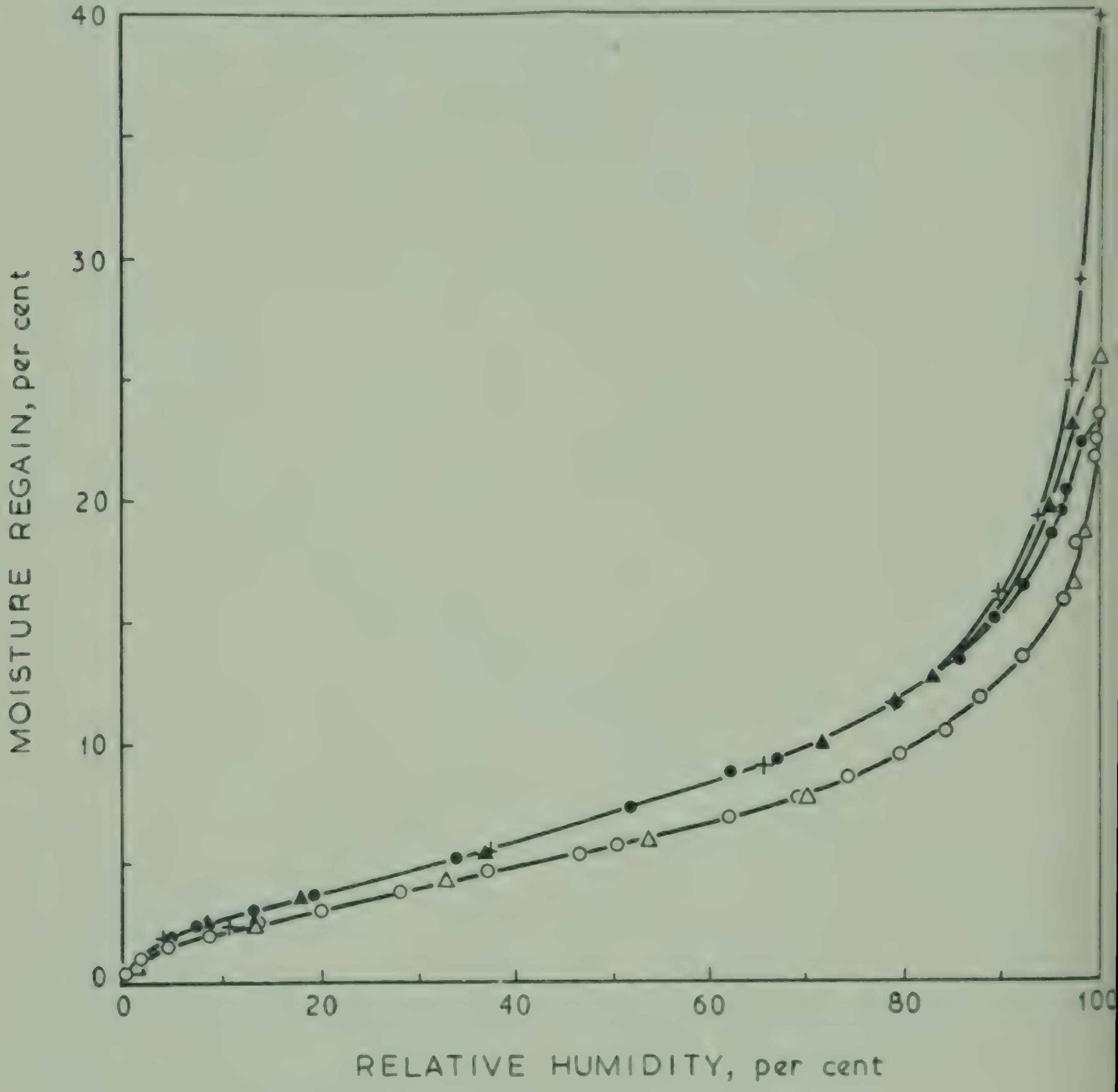

Figure 4. Sorption of water by scoured cotton at 25°

Although, as previously mentioned, our main concern is wit scientific principles, it is important to those who handle cellulos and starch that the practical consequences of this dependence o condition on prehistory should be borne in mind, particularly whe it is desired to condition a number of samples of unknown prehistor at any given humidity and temperature, for example, to compar their weights or to make physical tests on them. In order that th results shall be strictly comparable, the conditions of the sample must be representable by corresponding points on their equilibrium areas, and the only practicable way of ensuring this is to make the measurements in such a way that the representative points of the various samples shall all be on the boundary absorption curve at the test humidity, the absorption curve being preferred to the desorption because of the high-humidity variability of the latter referred to above. Figure 3 shows that in limited cycles the transition from one boundary curve to the other is effected, not by short direct paths, but by long curves similar in shape to the boundary curves themselves; hence in order to ensure that the samples shall be in comparable condition in an atmosphere of, for example, 65 per cent relative humidity, they should be exposed for about 6 hr in an atmosphere of not more than 10 per cent relative humidity before they are introduced into the test atmosphere.

Cause of Hysteresis

In considering a possible explanation of the phenomenon of hysteresis we need not enlarge on the usual picture of cellulose as consisting of both crystalline and amorphous regions, with gradual transition from one to the other, and with molecules so long in comparison with the length of the crystallites that one molecule may extend through more than one crystalline and more than one amorphous region. When cellulose is first formed, for example within the cotton fibre, it is in the presence of water, and the hydroxyl groups whose residual combining power is not satisfied by combining with one another will have water molecules attached. As the fibre dries, some of these groups will be freed, and the cellulose molecules will tend to rearrange themselves so that these hydroxyl groups may bond to one another. If the cellulose is dried over a desiccant this process will continue until all the water has been removed, though it will proceed with increasing difficulty as relative movement of the molecules is hindered by the bonds already formed. But, though there is tendency for this rearrangement to occur, it may not always occur; some molecules will be held together more closely than others, and a change of configuration will be possible only when a loosely held molecule attains momentarily to a sufficiently large amplitude of vibration. Hence the strengths of the inter-hydroxyl bonds will cover a fair range of strength, those formed when the amount of water was high and rearrangement easy will be strong, and those formed when the material is nearly dry will be much weaker. When this explanation was first put forward,[21] it was considered that completely dry cellulose might contain some free hydroxyl groups, but recent infra-red work has indicated[22] that the number of free hydroxyls can only be small under any condition This work has also confirmed, however, that the inter-hydrox hydrogen bonds in the amorphous regions do in fact vary i strength, so that no important alteration of the picture is required.

If the dry cellulose is now allowed to absorb water, the absorptio will occur in the first instance on any free groups that remain, an on those groups held together by bonds of lowest strength; th number of groups available for sorption will increase as absorptio proceeds, since a proportion of these, momentarily freed by therma vibration, will be prevented from recombining by the attachmen to them of water molecules. However, the increase in the number available hydroxyl groups, during absorption from one relativ vapour pressure to another, will be less than the correspondin decrease during desorption, since the amplitude of vibration of molecule held to another by bonding between one or more hydrox groups will be less than that of the same molecule in which thes groups are bound merely to water, without any cross-linking.

This explanation of hysteresis, therefore, postulates that durin desorption the number of hydroxyl groups available for the attachment of water decreases and during absorption it increases, bu with a lag during absorption, so that the same number of availabl hydroxyl groups corresponds to a higher vapour pressure in absorption than in desorption. It is, of course, only one possible explanation, and several others are available.[7,19,23] Most of these ar developed from a thermodynamic rather than a mechanistic background, and the authors of at least two of them[7,19] state that th explanations offered are not essentially different from that suggeste above; it is not proposed, therefore, to discuss them further.

The Effect of Temperature

The uptake of water is an exothermic reaction, and it consequently follows as a thermodynamic necessity that the amount water taken up at constant partial vapour pressure shall decreas with increasing temperature. The effect of temperature has bee studied in some detail for cotton, and Figure 5, which shows series of isotherms for the absorption of water by cotton cellulose temperatures in the range 10°–50°, provides experimental confirmation of the theoretical deduction.[24] Figure 6 shows[24] that in t range 50°–100° the same effect is found at humidities below abo 85 per cent R.H., but at higher humidities the curves cross o another, indicating that in this region the amount of water taken u at constant humidity *increases* with increasing temperature.

Before we attempt any explanation of this, two addition experimental observations are of interest. We have already noted that drying out at room temperature causes a temporary reduction in the amount of water sorbed, and that this reduction is removed by exposing the sample to a saturated atmosphere, so producing the hysteresis effect. But if a sample of cotton is dried for some time at a high temperature, for example 110°, its capacity for taking up water is reduced,[25] both absorption and desorption curves being lower than the corresponding curves for unheated cotton. On the other hand if it is heated in the presence of a high concentration of water vapour, under conditions such that the water cannot escape, its hygroscopicity may be increased.[21] Now the molecules of the cellulose, and in particular its hydroxyl groups, are in a state of thermal vibration that will increase with increasing temperature; this can result in both the making and the breaking of the bonds linking the hydroxyls to one another, and the final result will depend on the amount of water present. Where it is high, most of the hydroxyl groups will have water molecules attached to them, and hence the tendency for further cross-bonding to occur by mutual satisfaction of hydroxyl groups on any adjacent cellulose

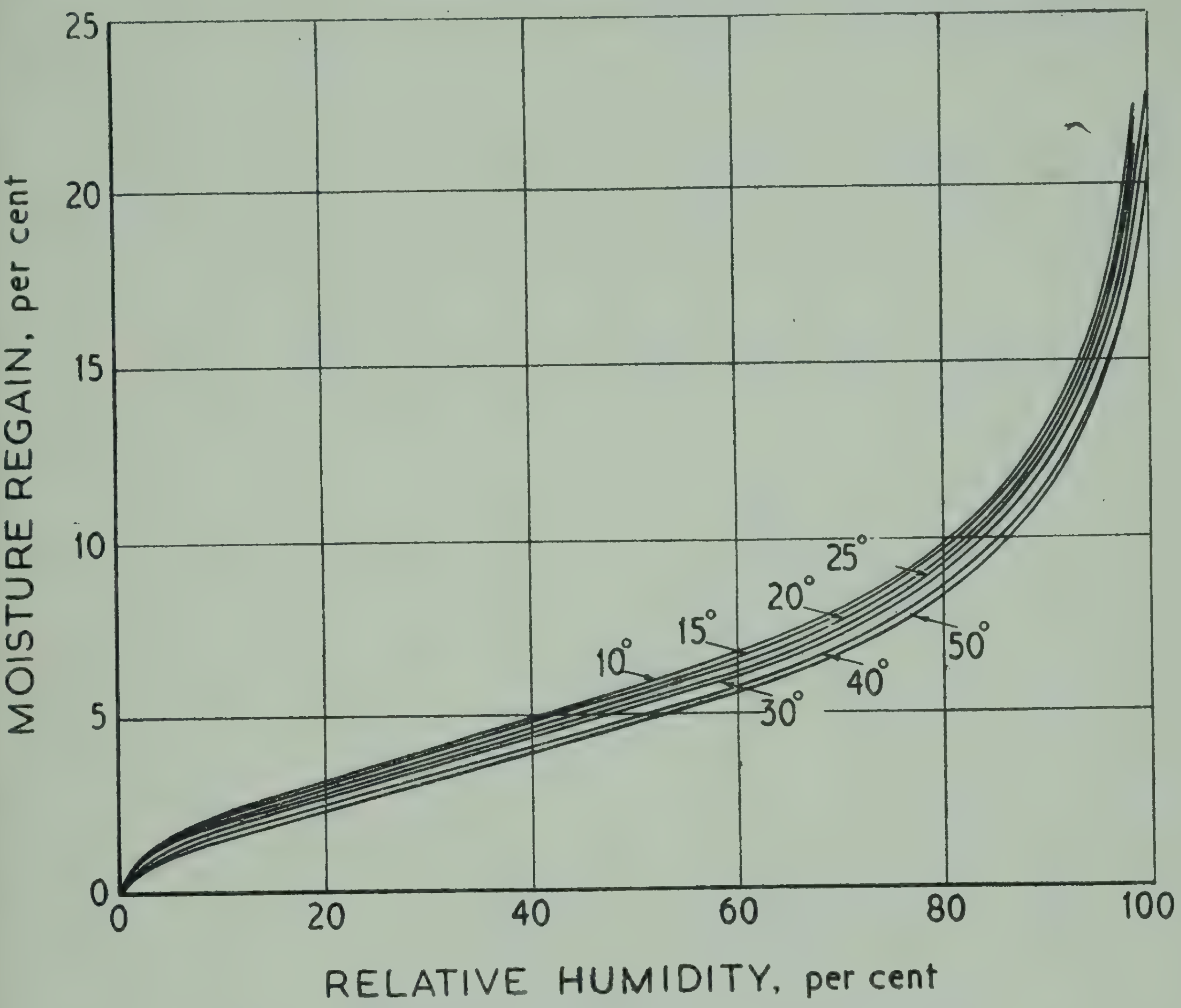

Figure 5. Absorption of water by scoured cotton, 10°–50°

molecules will be negligible. On the other hand, the breaking already existing links, which because of increased vibration will occ more readily at high temperature, may easily result in both t groups concerned failing to recombine, since water molecules w probably have attached themselves before recombination is possib The final result is a more or less permanent increase in the numb

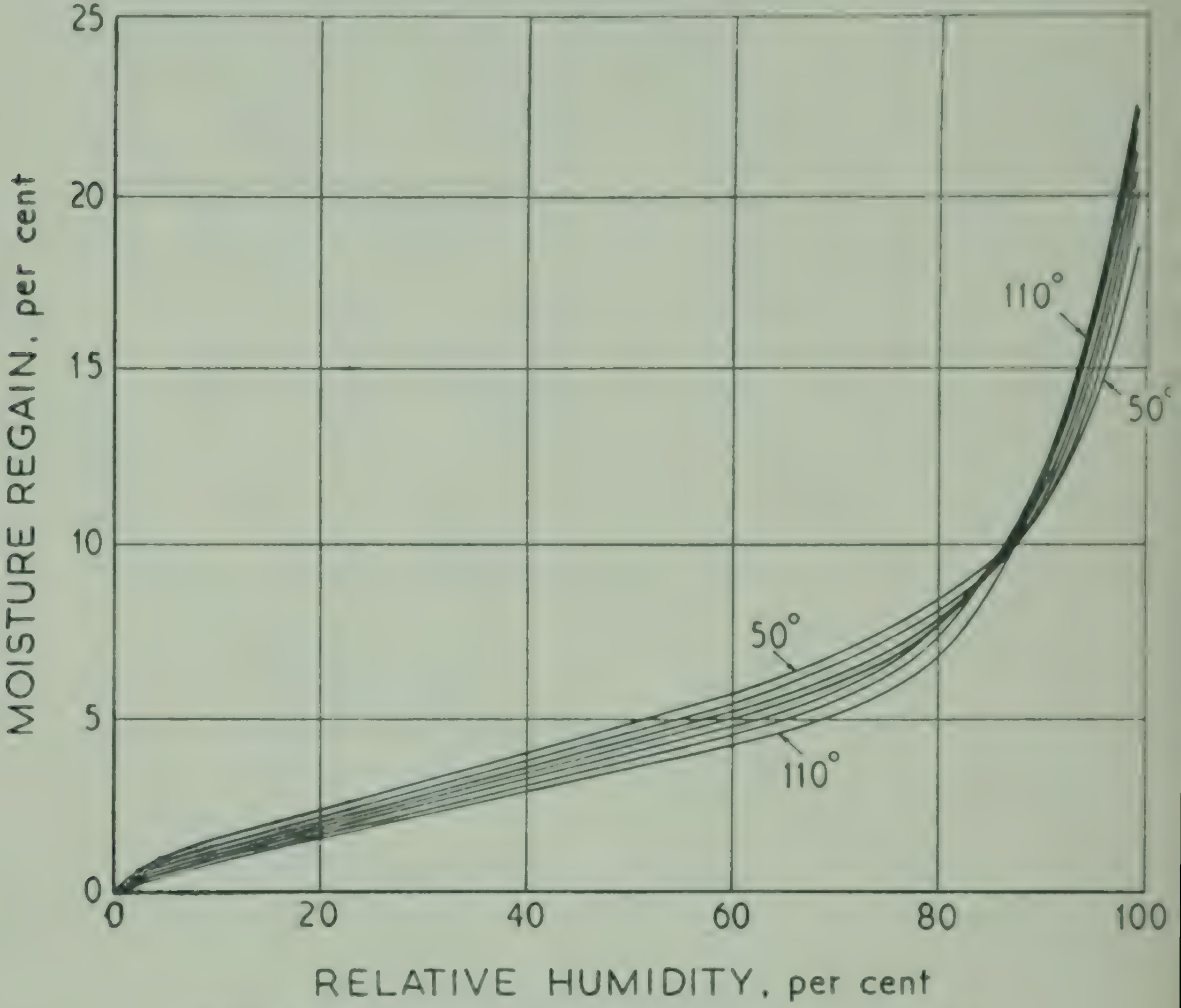

Figure 6. Absorption of water by scoured cotton, 50°–110°

of available hydroxyl groups, and hence in the subsequent hygr scopicity of the cellulose. However, if the amount of water prese is small the tendency for additional groups to become available w be more than counterbalanced by the tendency of existing availab groups to combine more strongly with one another, since few of the will have attached water molecules to prevent this happening. Th final result is therefore a permanent decrease of hygroscopicity.

Since heating in a saturated atmosphere increases the hygr scopicity of cotton, and heating in an atmosphere of low humidi decreases it, it is to be expected that at some point between the tw extremes no alteration will be produced. The existence of su points is suggested by the intersections of the high-temperatu isotherms, but it is more instructive to consider these from anoth point of view. The general effect of increasing the temperature is to decrease the moisture uptake of cotton in equilibrium with an atmosphere of constant relative humidity by decreasing the average amount sorbed per available hydroxyl group, as was shown by the curves for the temperature range 10°–50°. But if the temperature and the amount sorbed exceed certain values, the resulting increase in the number of available hydroxyl groups may be sufficient to counterbalance the decrease in the average amount attached to each group, so that the temperature effect reverses.

If this suggested explanation of the reversal of the normal temperature effect is accepted, then since it depends on an increase in the number of available hydroxyl groups the reversal would be expected to be less pronounced in materials which already have a large proportion of these groups available. We shall see presently that mercerized cotton has considerably greater availability than scoured cotton, and regenerated cellulose greater still; hence we would expect the reversal to be less pronounced for mercerized cotton, and very slight, or even non-existent, for regenerated cellulose. Unfortunately, there is so far no information available to check this, but it is hoped that the data will be forthcoming in the not-too-distant future. Since hysteresis has also been attributed to variation in the number of available hydroxyl groups, and the possibility of such variation is smaller the larger the number of groups permanently available, it would be expected that hysteresis would decrease with increasing temperature, particularly at higher humidities. There is evidence that this in fact happens,[24] and rather more scanty evidence that hysteresis might disappear entirely at about 100°.

The temperature effect of the swelling of cotton in water vapour is similar to that of the actual sorption, as is evident from Figure 7, the two curves of which represent respectively the swelling of cotton fibres in water and the sorption of water (extrapolated to 100 per cent R.H.), plotted against temperature;[26] the increased uptake of water at high temperatures and humidities is reflected in increased volume swelling. This is of considerable importance in textile processing, because when cotton is swollen it is more easily deformed. Hence, profound changes in properties can be induced by the application of pressure or tension during drying, and indeed by altering the rate of drying, which controls the length of time the material remains in a plastic condition and hence susceptible to external influences. The effect of drying conditions on, for example, the handle of cloth is well known to textile finishers, while its plasticity at high temperatures and humidities is utilized in various

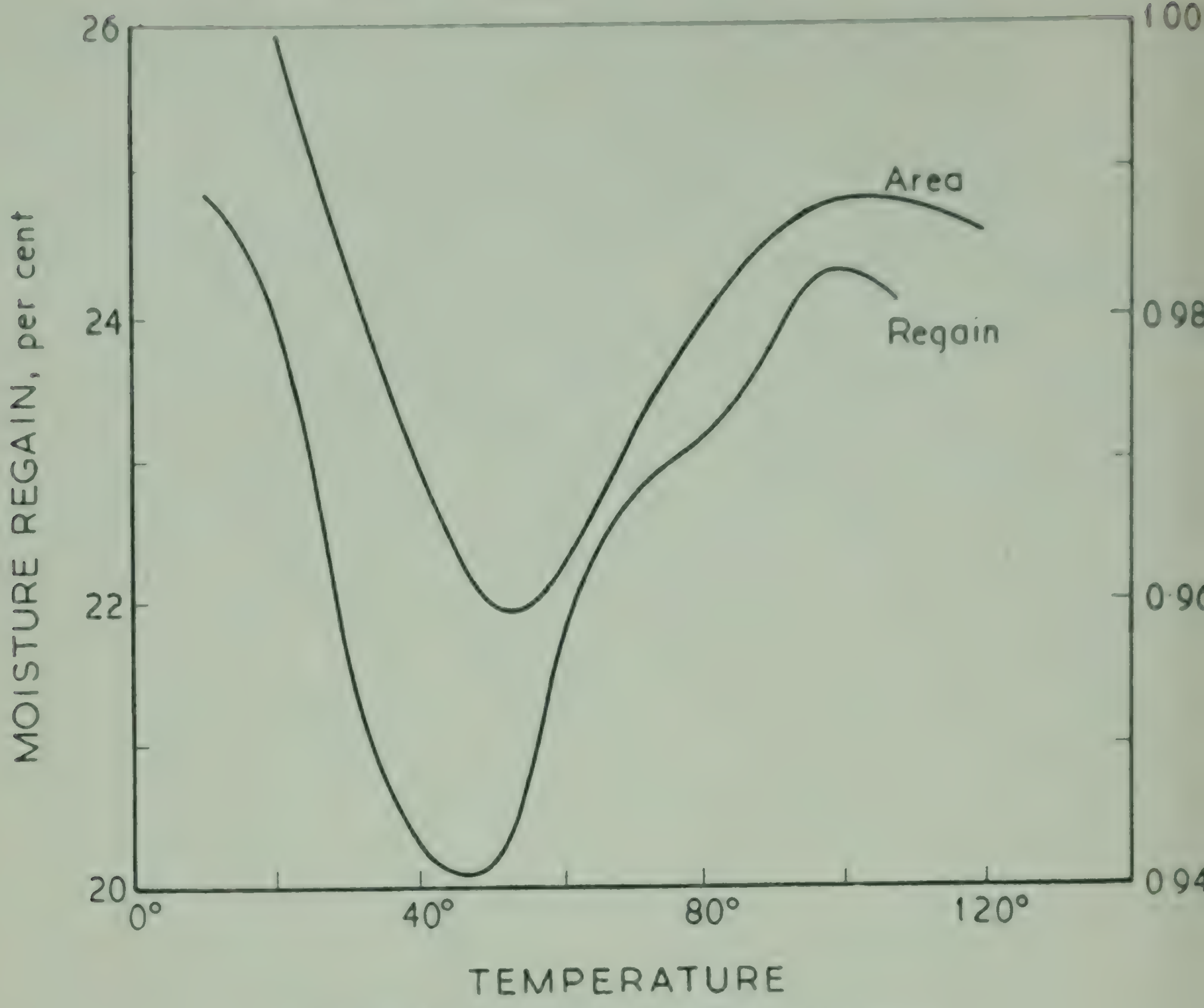

Figure 7. Cotton fibre: swelling and sorption

calendering processes. This kind of effect is even more pronounc during the drying of starch and gelatin solutions; recent work h shown that the properties of the dry film[27] can be greatly altered changes in the drying conditions.

Mercerized Cotton and Regenerated Cellulose

The technical processes that are normally applied to cellulo and in particular cellulose textiles, have in general a fairly sm effect on their capacity for sorbing water; with cotton, for examp the differences caused by most processes are smaller than the unc tainties that can be ascribed to unknown differences of prehistory There are, however, some important exceptions; in particul processes that cause large swelling of the fibre result in large increa of hygroscopicity. Possibly, the most important of these, a certainly that which has received most study from this point of vi is the process of mercerization. This involves steeping cotton other cellulose fibre in a solution of a caustic alkali, the concent tion of which must exceed a minimum value determined by fibre, the temperature, and the particular alkali used. An enorm lateral swelling of the fibre results, and usually the technical pro is conducted in such a way that the accompanying longitudinal shrinkage is either prevented or allowed to occur and then counteracted by stretching, but the most pronounced swelling action is achieved when the fibre is free to change its dimensions without any external restraint.

Figure 8 shows the isotherm for a sample of loose scoured cotton

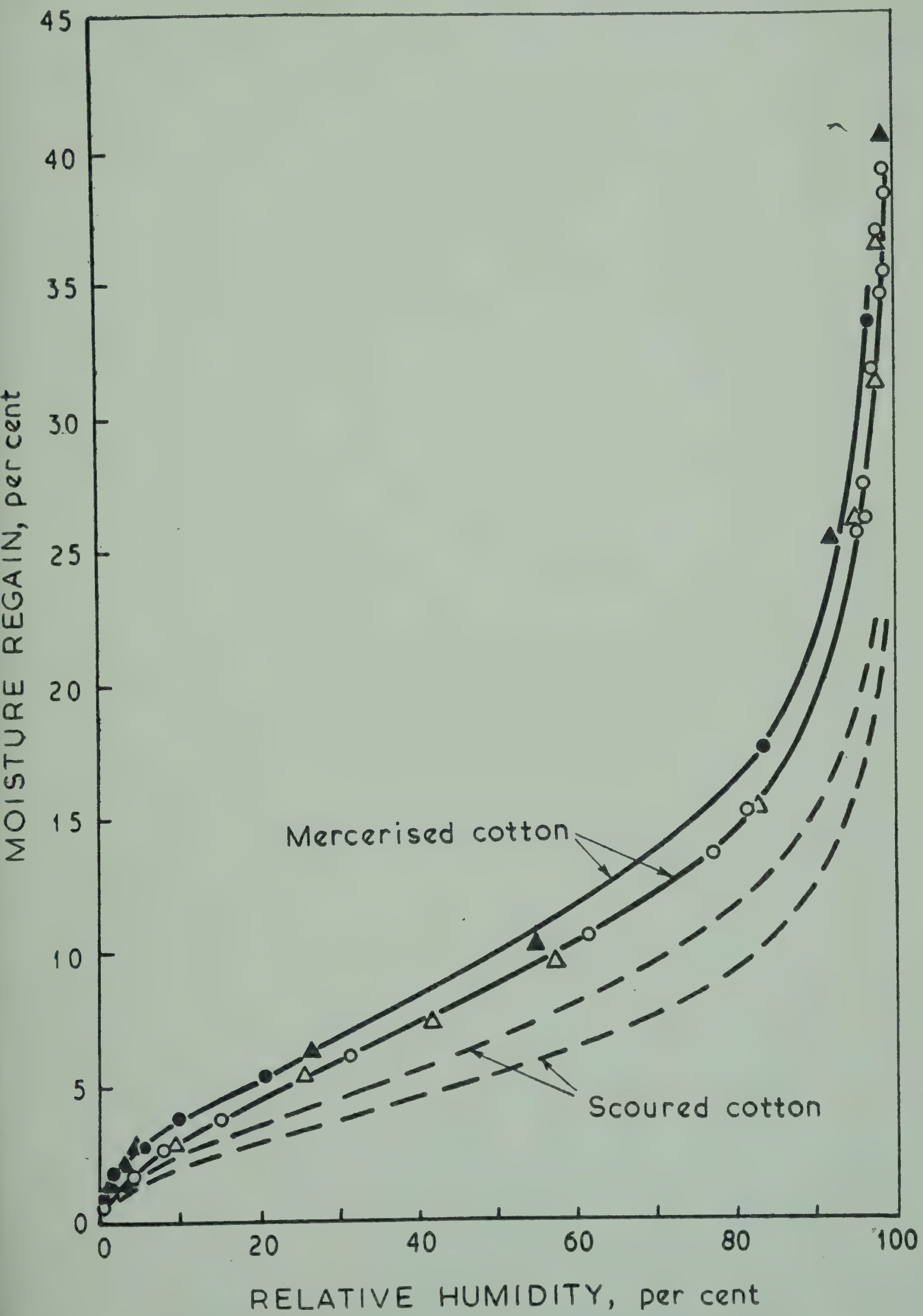

Figure 8. Sorption of water by mercerized cotton at 25°

that had been immersed in a 15 per cent solution of sodium hydroxide, washed, and dried.[29] The isotherm for the original unmercerized cotton is also shown, and it is obvious that a large increase of hygroscopicity has resulted from the treatment. The general shape of the curves is however the same; indeed the curves for the mercerized cotton can be obtained from those for the unmercerized by multiplying the ordinates of the latter by a factor that is pretty well independent of the humidity. This is shown by the data of Table 1,[29] which shows that the ratio of the moisture uptake of the mercerized cotton to that of the original scoured cotton is approximately constant. Almost certainly this is an approximation only—some variability can in fact be seen in the Table—and it may well be that, as suggested by Peirce,[8] only the closely-bound water should demonstrate the constant ratio, but for our present purpose the ratios of total water sorbed are sufficiently constant to justify the acceptance of their mean value as a measure of the change of sorptive capacity brought about by the mercerization process.

Table 2 provides further evidence of this approximate constancy of ratio for a series of samples treated with solutions of sodium hydroxide of different concentrations. It gives the absorption figures only, but the desorption results are similar. The effect of concentration is shown more clearly in Figure 9, where the amounts taken up at different values of the humidity are plotted against the concentration. There are two maxima in the curves; in itself this is of no special interest for our present purpose, but when the sorption curves are compared with those showing the swelling of the fibre in relation to concentration, we see that the sorption curves are similar in shape to those representing swelling. When swelling is hindered by the application of tension, sorption is correspondingly reduced.[30]

It is unnecessary to consider in detail what happens to cellulose during mercerization; it is sufficient to note that in the very great opening-up of the structure that occurs there is a large increase in the number of hydroxyl groups available for combining with water, dyes, and so on. Naturally the degree of swelling and the water sorption go hand in hand, both being due to the same primary cause. In mercerization, particularly the mercerization of loose cotton, the degree of swelling can be very large, but even so it falls far short of the complete disintegration of the structure that occurs when cellulose is dissolved, when all or nearly all the cross-links, including those in the crystallites, may be assumed to be broken. Because of this it would be expected that the number of available hydroxyl groups in a cellulose regenerated from solution would be

Table 1: Cotton mercerized in 15 per cent sodium hydroxide

ABSORPTION				DESORPTION			
Relative humidity (per cent)	Moisture regain (per cent)		Sorption ratio	Relative humidity (per cent)	Moisture regain (per cent)		Sorption ratio
	Mercerized	Soda-boiled			Mercerized	Soda-boiled	
5	2·07	1·41	1·47	95	26·85	18·30	1·47
10	3·00	1·98	1·52	90	21·06	15·00	1·40
15	3·72	2·46	1·51	85	17·97	13·11	1·37
20	4·41	2·82	1·56	80	16·23	11·76	1·38
25	5·07	3·21	1·58	75	14·82	10·53	1·41
30	5·82	3·66	1·59	70	13·62	9·60	1·42
35	6·54	4·05	1·62	65	12·57	8·73	1·44
40	7·23	4·47	1·62	60	11·64	7·92	1·47
45	7·92	4·92	1·61	55	10·74	7·26	1·48
50	8·70	5·37	1·62	50	9·93	6·69	1·48
55	9·45	5·82	1·62	45	9·06	6·06	1·49
60	10·23	6·30	1·62	40	8·22	5·52	1·49
65	11·10	6·90	1·61	35	7·44	4·98	1·49
70	12·06	7·56	1·60	30	6·69	4·50	1·49
75	13·08	8·31	1·57	25	5·88	3·96	1·48
80	14·46	9·39	1·54	20	5·13	3·48	1·47
85	16·20	10·56	1·53	15	4·38	2·97	1·47
90	18·75	12·30	1·52	10	3·66	2·46	1·49
95	23·49	15·15	1·55	5	2·67	1·71	1·56
			Mean 1·57				Mean 1·46

Table 2

Relative humidity (per cent)	ABSORPTION																
	Moisture regain (per cent)																
	Soda boiled	Mercerized with *n* per cent sodium hydroxide solution															
		n = 9·3	Sorption ratio	*n* = 13·4	Sorption ratio	*n* = 15·0	Sorption ratio	*n* = 17·5	Sorption ratio	*n* = 24·9	Sorption ratio	*n* = 36·6	Sorption ratio	*n* = 4·8	Sorption ratio	*n* = 20·0	Sorption ratio
10	2·14	2·24	1·05	3·35	1·57	3·42	1·59	3·39	1·58	3·58	1·67	3·10	1.45	—	—	—	—
15	2·53	2·69	1·06	3·94	1·56	4·08	1·61	4·03	1·59	4·25	1·68	3·71	1·47	—	—	—	—
20	2·98	3·14	1·05	4·59	1·54	4·74	1·59	4·70	1·58	4·94	1·66	4·32	1·45	—	—	—	—
25	3·33	3·55	1·07	5·17	1·55	5·36	1·61	5·31	1·59	5·65	1·70	4·88	1·47	—	—	—	—
30	3·74	4·00	1·07	5·79	1·55	5·98	1·60	5·92	1·58	6·30	1·68	5·47	1·46	—	—	—	—
35	4·14	4·45	1·08	6·42	1·55	6·64	1·60	6·59	1·59	7·01	1·69	6·05	1·46	—	—	—	—
40	4·57	4·93	1·08	7·09	1·55	7·31	1·60	7·26	1·59	7·71	1·69	6·67	1·46	4·62	1·01	7·18	1·57
45	5·02	5·42	1·08	7·76	1·55	8·00	1·59	7·95	1·58	8·42	1·68	7·31	1·46	5·08	1·01	7·8	1·55
50	5·52	5·95	1·08	8·50	1·54	8·69	1·57	8·65	1·57	9·15	1·66	8·00	1·45	5·54	1·00	8·5	1·54
55	5·98	6·51	1·09	9·26	1·55	9·46	1·58	9·39	1·57	9·92	1·66	8·69	1·45	6·07	1·02	9·2	1·54
60	6·50	7·07	1·09	10·03	1·54	10·18	1·57	10·13	1·56	10·75	1·65	9·45	1·45	6·56	1·01	10·0	1·54
65	7·06	7·65	1·08	10·86	1·54	11·04	1·56	10·99	1·56	11·60	1·64	10·21	1·45	7·14	1·01	10·8	1·53
70	7·68	8·30	1·08	11·74	1·53	11·95	1·56	11·89	1·55	12·51	1·63	11·08	1·44	7·80	1·02	11·85	1·54
75	8·43	9·02	1·07	12·83	1·52	13·02	1·54	12·94	1·54	13·46	1·60	12·08	1·43	8·59	1·02	12·9	1·53
80	9·42	10·03	1·06	14·21	1·51	14·46	1·54	14·37	1·53	15·20	1·61	13·58	1·44	—	—	14·2	1·51
85	10·80	11·41	1·06	16·13	1·49	16·45	1·52	16·27	1·51	17·25	1.60	15·14	1·40	—	—	16·0	1·48
90	12·53	13·06	1·04	18·58	1·48	18·93	1·51	18·80	1·50	19·97	1·59	17·35	1·38	—	—	18·9	1·51
95	15·23	15·87	1·04	22·64	1·49	23·58	1·55	23·04	1·51	24·88	1·63	21·04	1·38	—	—	23·55	1·58
100	23·76	25·89	1·09	36·91	1·55	37·79	1·59	37·34	1·57	39·23	1·65	34·16	1·44	—	—	—	—
		Mean	1·07	Mean	1·53	Mean	1·57	Mean	1·56	Mean	1·65	Mean	1·44	Mean	1·01	Mean	1·54

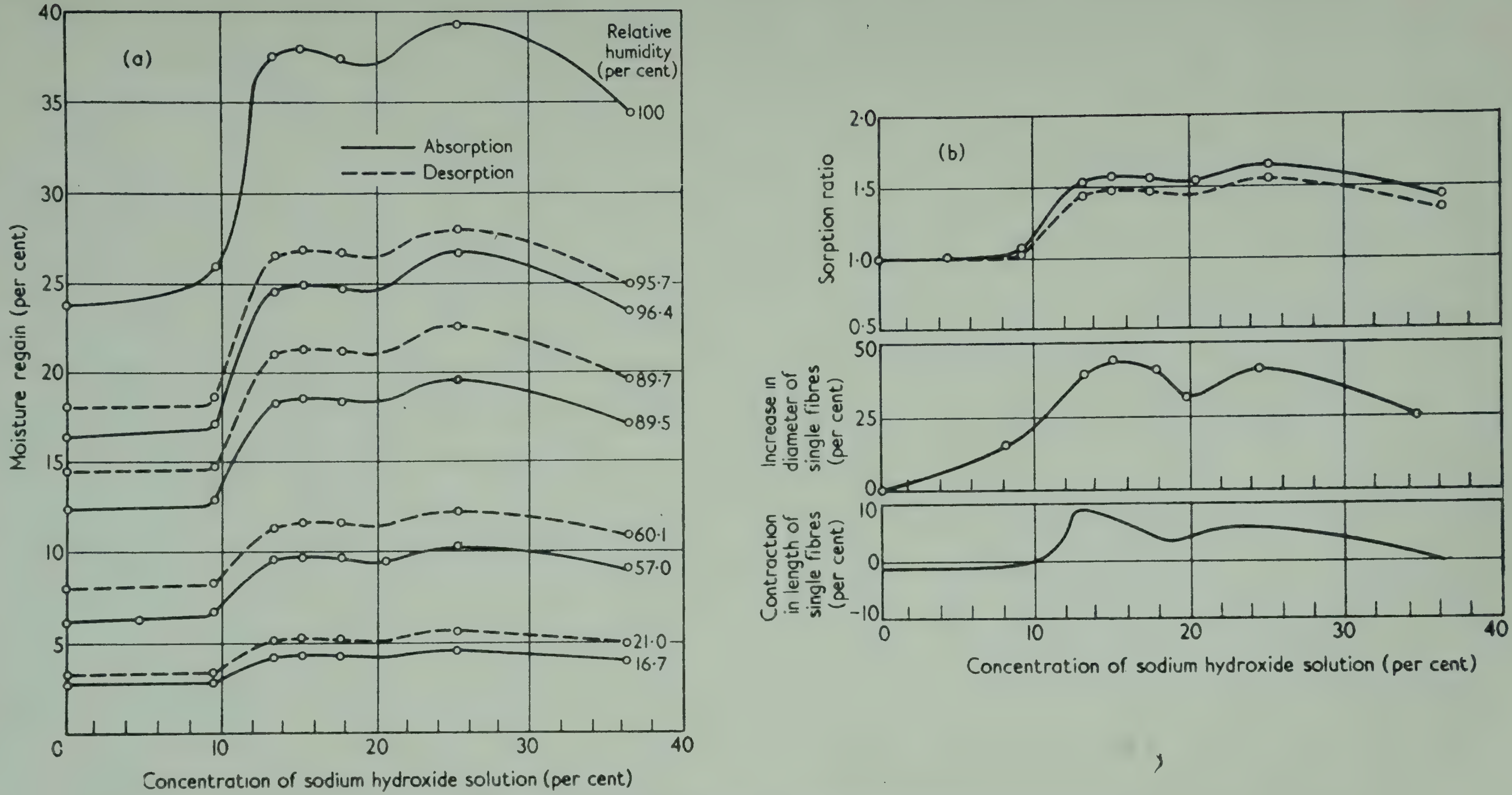

Figure 9. Sorption of water by cottons mercerized at different concentrations

much greater than even in a mercerized cotton produced wit maximum swelling. This, in fact, has been found; the isotherms fo the regenerated-cellulose rayons are of the same shape as those fo scoured cotton, and can be derived from the latter, at least approximately, by multiplying all the sorption values by the appropriat sorption ratio. But the actual values of these ratios are greater tha have been found for any mercerized cotton, being generally withi the range 1·8–2·2, as compared with about 1·6 for the most highl swollen mercerized cotton.

The fact that a large increase in the number of available hydroxyl groups occurs in the processes of mercerization and regeneration is shown quite clearly by a closer study of water sorption. If a sample of cotton after withdrawal from the mercerizing liquor is washed and its desorption curve determined from the wet condition the isotherm shown in Figure 10 is obtained.[20] From this it is clear that cotton immediately after mercerizing, before it has even been air-dried, is capable of holding very large amounts of water. But, in this condition, the number of available hydroxyl groups is so large that as the material is dried many of them become permanently

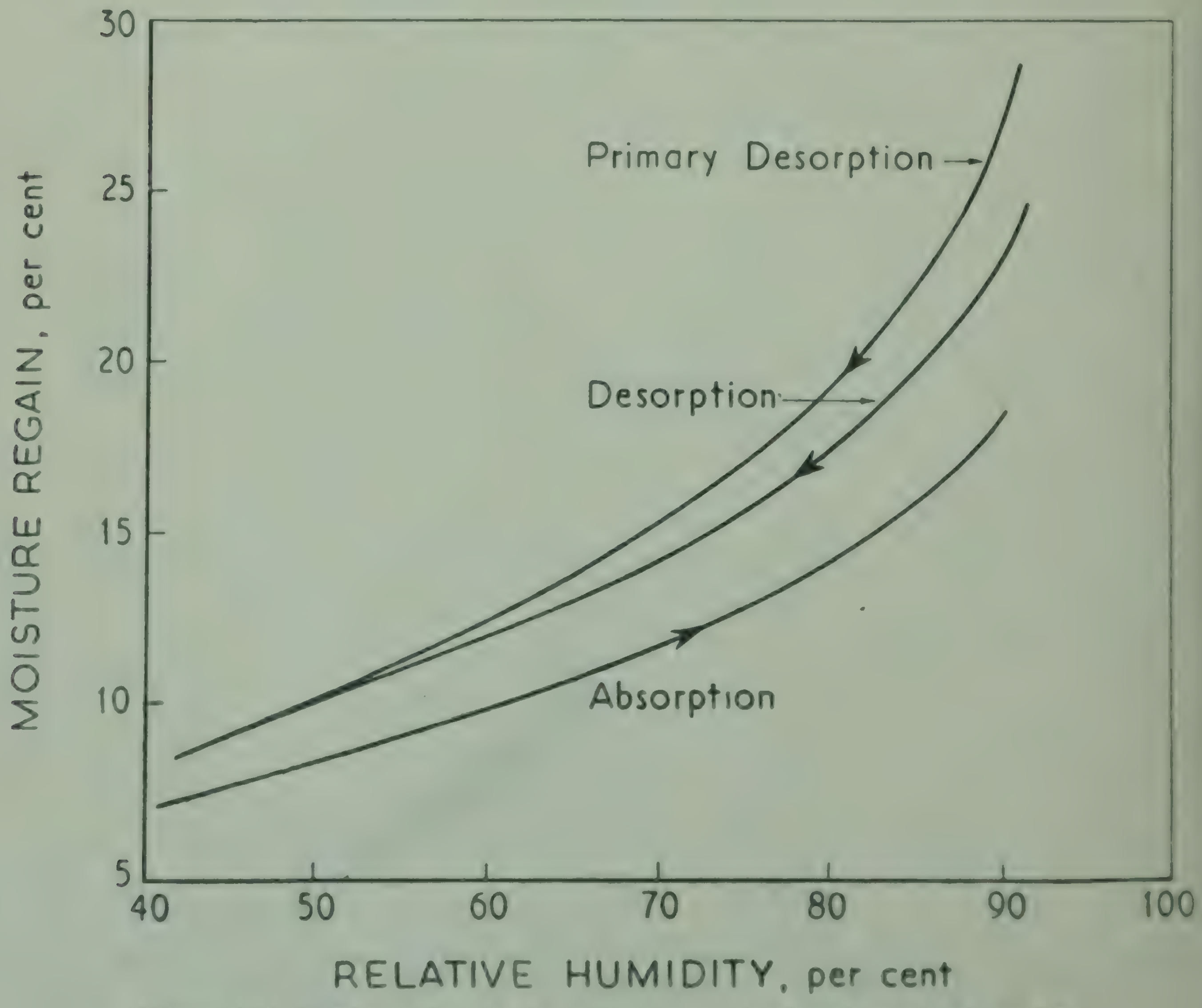

Figure 10. Sorption of water by cotton, mercerized and not dried, at 20°

linked to one another, so that they cease to be available as potential sorbing points. This is no doubt the reason why the uppermost curve of this figure—the primary desorption curve—is irreproducible, and why the subsequent desorption curve obtained after the material has been dried shows that much of the original hygroscopicity has been lost. The same kind of effect is observed in cotton fibres taken from the cotton boll before it has opened to allow the fibre to dry for the first time, and in even greater degree in regenerated cellulose while it is still wet; this is one of the reasons why ' never-dried ' regenerated cellulose film is so much used in research laboratories when it is desired to study cellulose in its most accessible and most reactive form.

CELLULOSE DERIVATIVES

The large difference in the water solubility of cellulose and starch is ascribable to differences in their fine structures. At first sight it appears anomalous that cellulose should be completely insoluble in water and other hydroxylic solvents, while starch, cellulose ethers, and acetates of low degree of substitution are soluble in water. That the highly substituted ethers and acetates are insoluble in water but soluble in benzene and methylene chloride respectively is confirmation that the substituent groups are essentially hydrophobic. Why then should the introduction of a few hydrophobic groups into a water-insoluble substance confer water-solubility on it? Closer examination reveals[31] that the real anomaly is not in the water solubility of the lowly-substituted derivatives, but in the insolubility of the cellulose itself, and that two features operating together are responsible for this.

The first is that the inter-hydroxyl bonds uniting the cellulose molecules to one another in the crystallites are strong, and hence that, although those portions of the molecule situated in amorphous regions may be regarded as dissolved in the water that combines with the hydroxyl groups there, it is the crystallites themselves that must be broken up before dissolution can be achieved. But that consideration, though necessary, is not sufficient to cause insolubility. Polyvinyl alcohol also exists in partially crystalline form, and the bonds uniting the molecules in the crystallites are also the strong ones between hydroxyl groups, yet polyvinyl alcohol is soluble in water. The reason for this is that the molecule of polyvinyl alcohol is a flexible one, so that dissolution of a molecule from a crystallite can be achieved by a peeling action involving the breaking of only one hydrogen bond at a time. The presence in cellulose of an inflexible ring structure carrying several potential cross-linking groups makes it necessary for all the cross-bonds to be broken simultaneously before part of the molecule can be bent out of the way to facilitate attack on the next segment, and the probability of this occurring is very small.

However, since it is both these features together that cause insolubility, the absence of any one of them will induce solubility So long as cellulose remains cellulose the ring structure and hence the rigidity are invariant. It is relatively easy to prevent crystallization, however, and there is little doubt that it is the presence of a few randomly situated substituent groups that makes some methy celluloses and cellulose acetates soluble in water by eliminating tha regularity of structure on which the possibility of crystallizing depends. It is to this that is attributable the anomaly that the replacement of a few hydrophilic groups by more hydrophobic ones can convert a water-insoluble material into a water-soluble one. When more such groups are introduced, the essentially

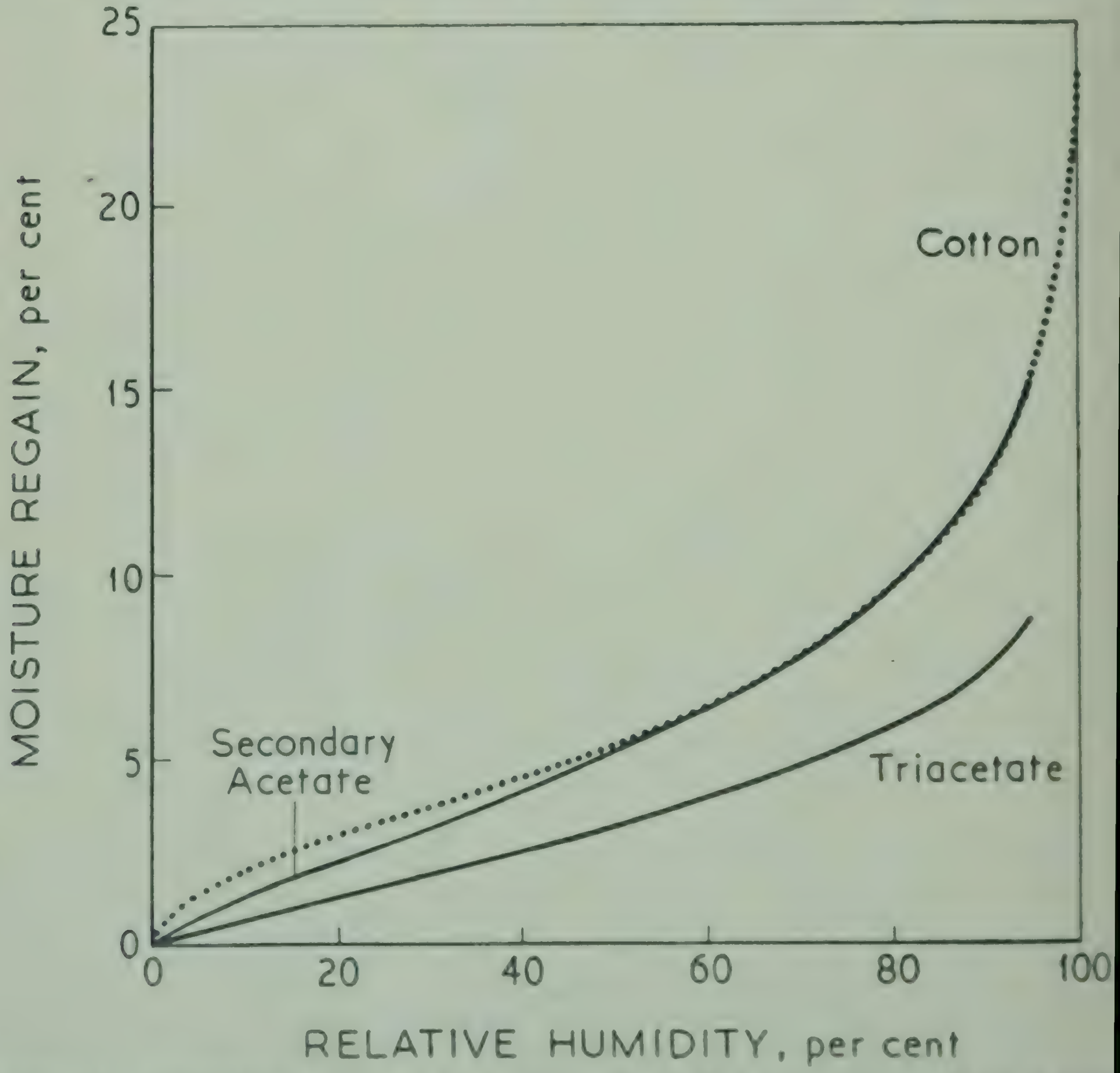

Figure 11. Absorption of water by cotton, secondary cellulose acetate, and cellulose triaceta

hydrophobic character of the substituent groups begins to take charge, and the derivative loses its solubility in water and becomes soluble in organic solvents.

The sorption of water by cellulose derivatives can now be briefly considered. Figure 11 shows isotherms for cotton, secondary cellulose acetate, and cellulose triacetate fibres, and shows how the initial bulge in the curve corresponding to the strong sorption tends to disappear as the number of hydroxyl groups in the molecule decreases.[1,32] These commercial materials are, however, relatively highly substituted, and it is more interesting to consider the relative sorptions of samples of different degrees of substitution (DS); for this purpose Figure 12 is of interest.[2,33] Curve 1 shows the moisture

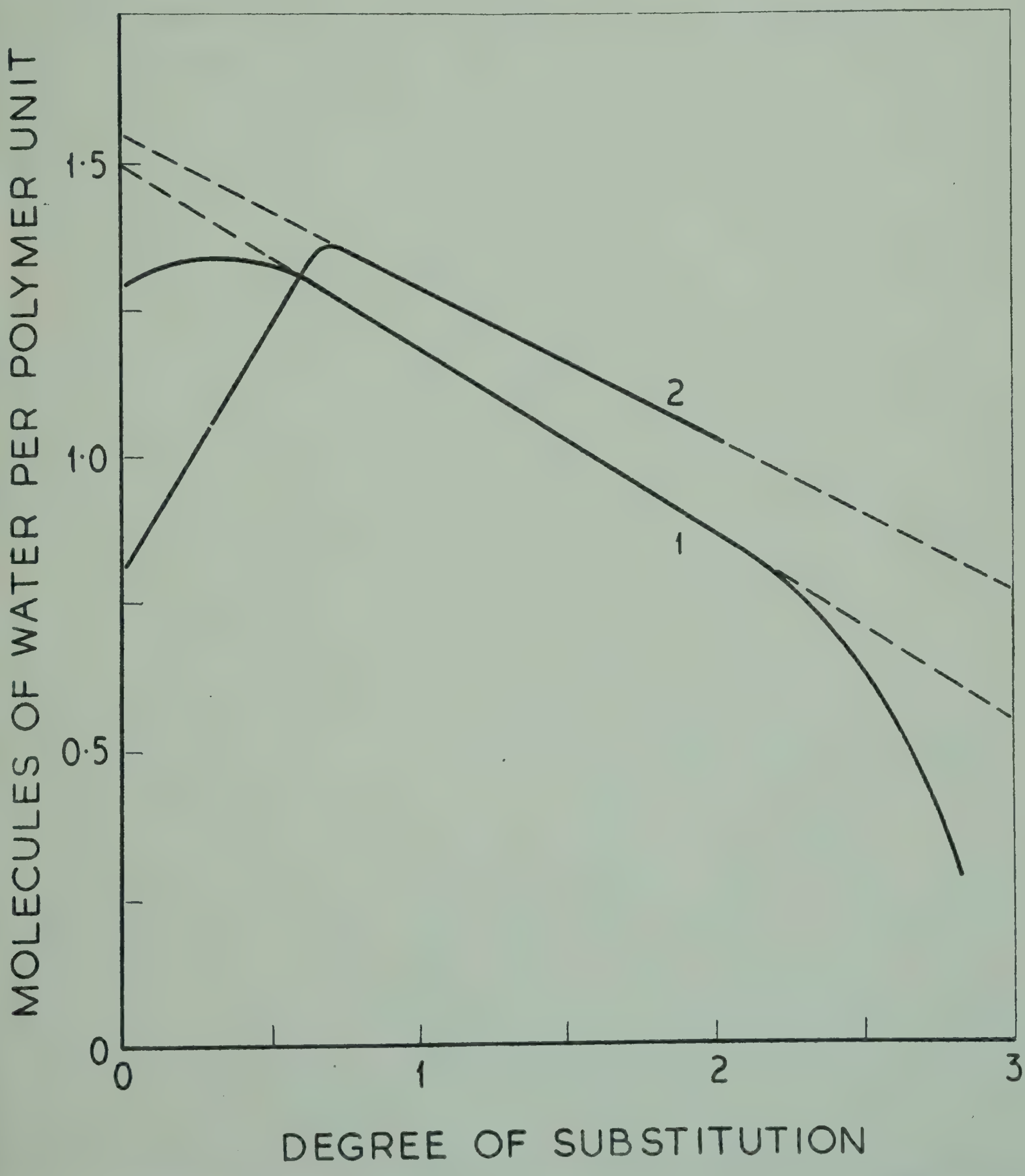

Figure 12. Absorption of water by methyl celluloses and cellulose acetates of different degrees of substitution

sorption at 65 per cent R.H. of a series of methyl celluloses prepare from a regenerated cellulose; the sorption rises to a maximu from the relatively high value of the regenerated cellulose itsel and falls linearly as the DS increases, falling more rapidly as the D approaches 3 and the contour of the molecule becomes sufficientl regular to permit crystallization once more. Curve 2 is for cellulos acetates and is of the same general shape, but starts at the lowe value characteristic of cotton and rises to a higher value because o the greater hygroscopicity of the acetyl groups as compared wit the methyl. On this basis, the intermediate linear portion may b regarded as representing the variation with DS of the moistur sorption of completely amorphous material, and the extrapolatio of this line to a DS of 0 should give the sorption value for complete amorphous cellulose, and to a DS of 3 for the completely amorphou derivative. The value obtained for cellulose is the same, as it shoul be, for both derivatives, and is about 17 per cent. Hence the actua sorption of any cellulose at 65 per cent R.H. divided by 17 will b the fraction of the total hydroxyl groups in that cellulose which ar accessible to water vapour. The values so obtained are not identica with the fractions of crystalline material, because the degree o accessibility so calculated includes hydroxyl groups on the crystallit surfaces, and the number of these will vary with crystallite size. Bu when a correction is made for this the values of degree of crystallinit obtained are in reasonable agreement with those obtained by othe methods, which may be taken as confirmation of the soundness o this interpretation.

STARCH

It has already been hinted that the amount of information availabl about the sorption of moisture by starch is much less than that fo cellulose. Probably part of the reason for this is that with starc films—the form that is of greatest industrial interest—the proble is even more complicated than with cellulose, since their investigation demands that consideration be given to the concentration of th original starch solution, the length of time it was heated, the rat and general conditions of drying, and various other preparativ factors that Nature has attended to for cellulose. Perhaps becaus of this much of the information available concerns studies of th moisture relations of starch granules before swelling, and henc tends to relate to commercial transactions and production contro rather than to a more fundamental study of structure.

The earliest systematic investigation of the sorption of water b raw starch was probably that of Rakovski[34] nearly fifty years ag and this was followed by that of Farrow and Swan[35] on starch films about a decade later. The information obtained in these investigations may be summarized as follows:

(1) The shape of the isotherms is the same as those for cotton, but corresponds to appreciably higher amounts of water sorbed;
(2) Hysteresis is found, and decreases with increasing temperature, probably disappearing at about 90°;
(3) The hygroscopicity of starch films is decreased by high-temperature heating;
(4) The effect of temperature on sorption is similar to that found with cotton, increasing temperature generally causing a reduction in the amount sorbed at constant relative humidity, but with a reversal of this effect at high temperatures and humidities.

These results are very similar to those obtained for cellulose, and later work has indicated that the resemblance is even closer. Thus some processes applied to starch produce modified starches which yield isotherms obtainable from that of the original starch by multiplying each ordinate by a ratio that is independent of the humidity.[36] This is analogous to mercerization, though the industrial processes applied to starch generally result in a decrease rather than an increase of hygroscopicity. Thus some British Gums, produced by heat dextrinization, had a sorption ratio of about 0·9, completely independent of humidity. More vigorous treatments, at 180° and 200°, of a series of starches reduced their original sorptions at about 40 per cent R.H. of 15–17 per cent to 12–14 per cent and 8–11 per cent respectively,[37] but there are no data at other humidities to show whether the ratio remains independent of humidity for such gross modification. Modified starches have also been produced by acid hydrolysis with mean ratios of about 0·9, though for such materials the ratio appears to be much less constant. Oxidized starches, on the other hand, have hygroscopicities little different from that of the original.

In spite of the relative paucity of the available information, there is sufficient to indicate that the general ideas that have been advanced for cellulose are also applicable, with suitable modifications, to starch. It is, for example, reasonably certain that the water molecules combine with the hydroxyl groups of the starch molecule by hydrogen bonding, and suggestions have been made[38] about the different ways in which the bonding can be achieved. However, a good deal of additional work will have to be done before a picture comparable in detail with that available for cellulose can be constructed.

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10

THE DEGREE OF POLYMERIZATION OF CELLULOSE AND STARCH

CHOICE OF SOLVENTS

The first difficulty confronting the investigator in this field is the lack of suitable solvents for cellulose, amylose, and amylopectin. The principal solvents for cellulose are cuprammonium and cupriethylenediamine; the components of starch can be molecularly dispersed in ethylenediamine or solutions of strong alkalis;[1] but unfortunately all three polymers are susceptible to atmospheric oxidation and degradation in alkaline solutions. Time-consuming absolute measurements of degree of polymerization (DP) are therefore at a disadvantage, and it is desirable to convert celluloses into their trinitrates and amyloses and amylopectins to their triacetates; these derivatives are soluble in neutral organic solvents. The preparation of the nitrates with mixtures of either nitric acid–phosphoric acid–phosphorus pentoxide or nitric acid–acetic acid–acetic anhydride is by far the simplest technique, but since amylose and amylopectin are more easily hydrolysed than cellulose it is usual to convert them into triacetates with a mixture of pyridine and acetic anhydride.

Proof that these conversions can be made without alteration to the DP is simple for the triacetates; they can be repeatedly deacetylated and reacetylated without change in intrinsic viscosity. The same method cannot be applied to the nitrates because during denitration the glucose ring tends to disintegrate. However, substantially the same DP can be obtained when the conditions of nitration are widely varied[2] and, as we shall see later, this circumstantial evidence has been confirmed by determinations of the DP of molecularly homogeneous fractions of cellulose acetate and of the corresponding nitrate prepared by deacetylation and nitration.[3]

THE DETERMINATION OF DEGREE OF POLYMERIZATION

The principal methods at present used to determine the molecular weight, and hence the DP, of high polymers involve measurements of osmotic pressure, light-scattering, sedimentation (equilibrium or sedimentation (velocity) in conjunction with diffusion. Unfo tunately, the expensive equipment, the immense amount of tin required, and the difficulty of correcting for non-ideality a obstacles to extensive sedimentation studies. Light-scatteri techniques do not suffer these disadvantages to the same exten but there are difficulties with calibration, dissymmetry correctio and in the preparation of optically clear solutions of natural su stances which, in contrast to synthetic high polymers, cannot obtained in a very pure state. Theoretically light-scattering metho are much the more powerful tool; they give much more informati about the shapes of molecules in solution than is given by sedime tation work and, in direct contrast to the osmotic method, t quantity measured increases with increasing DP. However, t osmotic method rests on an unassailable thermodynamic basis, an as it is the only method that is within the resources of the avera laboratory it will be dealt with in greater detail.

There are a considerable number of osmometers to choose fro but one in particular has never received the attention which deserves.[4] This osmometer consists of two metal blocks with matc ing concentric grooves for cells and differs from the Fuoss–Mea pattern in the following features. Polythene sleeves are used to ma metal-to-glass connections; they do not leak and they are mo robust than metal-to-glass seals. The provision of two capillari to each cell makes emptying and filling operations as simple as wi osmometers fitted with valves, without the annoyance of leakages which valves are prone. The principal advantage of all-gla construction—the ease with which trapped air-bubbles can seen—is also equalled, in that the liquid levels in each pair capillaries differ if air bubbles are present. Three other featur are unique and worth noting. In osmometers, the membrane a as a seal between the two cells but, as Higginbotham[4] pointed o the seal is more permanent and more satisfactory if metal spaci pieces are used to prevent unlimited compression and membra creep. Secondly, before assembly between the cells, the membran which is usually undried Cellophane, is mounted in a circul frame while still swollen with water, and when the water is displac by an organic solvent the membrane is stretched taut. The fra and membrane are mounted in the osmometer, and membrane fl is virtually eliminated. Thirdly, loss of solvent from the cells diffusion through the plane of the membrane is prevented enclosing the membrane frame in a sealed compartment filled wi the solvent.

The principal sources of error in osmotic pressure measurements—leakages, variations in capillary bore, and air-bubbles trapped at the entrances to capillaries—are well known. But it is not sufficiently appreciated that unless precautions are taken the limits of accurate measurements of an osmotic head may be fixed by errors of parallax and deviations arising from refractions at interfaces between air, glass, and water (in the thermostat tank), and not by the sensitivity of a cathetometer. Failure to eliminate these four sources of error is, in the author's opinion, the usual cause of the so-called ' cell constant ' or asymmetric membrane behaviour. It is also inadvisable to determine degrees of polymerization from osmotic pressure measurements at only three or four concentrations, particularly if the DP is high. The standard deviation σ may be reasonably small, but it should be remembered that the probability level of this estimate of error is extremely low. For a measurement based on observations at four concentrations a more realistic estimate would be $\pm$ 4·3 σ which corresponds to the 95 per cent confidence limits of the *t*-distribution.

Until about 1950, the osmotic head was almost always regarded as the difference in height between the solvent and solution menisci. However, as was realized by Boissonnas and Meyer[5] in 1937, the osmotic head measured in this manner is dependent on the vertical distance of the solvent meniscus from the centre of the membrane, since the columns of solvent and solution are of unequal density. The difference in height between the menisci is slightly less than the true osmotic head by an amount $h_0 - (h_0 \rho_0)/\rho_s$, where h_0 is the approximate distance between the centre of the membrane and the solvent meniscus, and ρ_0 and ρ_s are the densities of the solvent and solution respectively.

The effect that this correction has on the calculated DP depends considerably on the dimensions of the osmometer, and on the solvent–solute system being studied. With an osmometer of the type discussed above, the differences shown in Table 1 were obtained with cellulose trinitrate fractions in *n*butyl acetate.[3] It may be seen that the correction has a considerable effect if the DP is relatively high, and is only negligible below a DP of 200. On the other hand

Table 1: Effect of density correction on the calculated DP

DP, no density correction ...	2,106	1,974	1,741	1,477	1,347	981	682	443	236	109
DP, after density correction ...	1,782	1,631	1,521	1,264	1,202	840	610	420	228	107

some workers[6-8] are uncertain of the validity of the density correction, particularly when the membrane is in the vertical, but unt the correction was made Higginbotham (unpublished work) actuall observed negative osmotic heads with solutions of acetylate amylopectins of exceedingly high DP.

RELATION BETWEEN INTRINSIC VISCOSITY AND DEGREE OF POLYMERIZATION

Although the osmotic method of determining a DP involves n insurmountable experimental difficulties in the molecular weigh range 10^4–10^6 it is, nevertheless, too tedious a method if larg numbers of molecular weights are to be determined. Use is therefor made of the relation between intrinsic viscosity $[\eta]$ and DP. Fo most polymers this relation takes the form

$$[\eta] = K\,(\mathrm{DP})^{\alpha}$$

where K and α are constants for a given solute, solvent, and temperature. Compared with an absolute determination of DP, th determination of $[\eta]$ is both simple and rapid, but unfortunatel there is no agreement in the literature as to the values of K and for a particular polymer–solvent system.

The fundamental difficulty lies in the fact that a unique relatio between $[\eta]$ and DP can exist for molecularly heterogeneous hig polymers only when $[\eta]$ and DP, which are determined by statistica averages of the molecular length, relate to the same parameter o the molecular length distribution. In general, intrinsic viscosity i only strictly comparable with a viscosity-average DP and n absolute measure of DP yields this type of average. In particula the osmotic method yields a number-average DP, which may b anything down to one-third or even one-quarter of the viscosity average, depending on the DP distribution, so that correspondin measurements of $[\eta]$ and DP must be made on molecularly homogeneous fractions. Unfortunately, a completely homogeneou fraction cannot as yet be obtained, but by ensuring that the fractionation is as efficient as can at present be devised, errors from thi source are not serious.[9] From this point of view, sedimentation o light-scattering studies are advantageous since they can yield weight-average, or an average close to a weight-average, which i much nearer to a viscosity-average than is the number-averag In fact when $\alpha = 1$ the viscosity and weight-averages are identica Nevertheless, the experimental difficulties with these techniqu combine to outweigh the advantage of measuring an average D that is directly comparable with $[\eta]$ and, as we shall see later, suc measurements have yielded results no more consistent than the osmotic method.

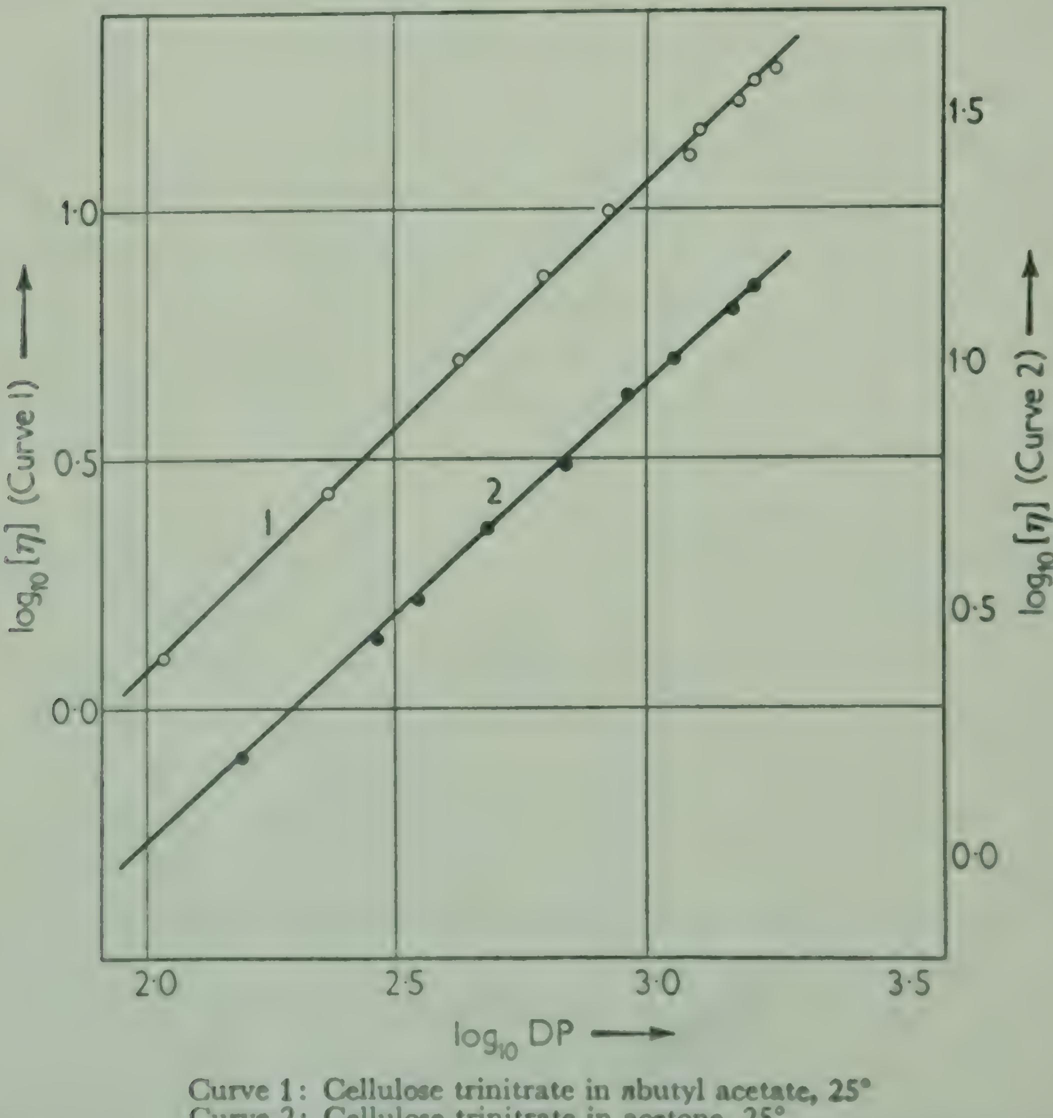
There are, however, two other difficulties. Firstly, the intrinsic viscosity is dependent on the degree of substitution of derivatives. It is essential, therefore, to ensure that the intrinsic viscosity of a particular sample is not used to calculate a DP from a relation based on a different degree of substitution. Large errors may arise from this source; for example, the intrinsic viscosity of cellulose nitrate increases by about 60 per cent when the nitrogen content is increased from 12·0 to 14·14 per cent (theoretical trinitrate).[10–12] Secondly, when the DP is relatively high the viscosities of the solutions, from which the intrinsic viscosities are computed, are strongly dependent on the rate of shear or shear stress operating during their measurement. The level of DP at which this difficulty arises varies; for molecularly homogeneous cellulose trinitrate fractions it becomes appreciable[13] at a DP of about 2,000; for fractionated cellulose and fractions of partially substituted cellulose derivatives, the critical DP will be higher since their molecules are more flexible and less susceptible to orientation during flow. If the polymers are molecularly heterogeneous, still more caution is called for, because the average DP gives no indication of the maximum chain-length present. The intrinsic viscosity may be reduced by as much as 25 per cent by shear effects. Attempts to avoid the difficulty of extrapolating viscosity data to zero rate of shear or zero shear stress by calculating the intrinsic viscosity from viscosity data obtained with capillary viscometers and interpolated at a so-called standard mean rate of shear are to be deplored; such a quantity cannot be defined for non-Newtonian fluids in capillary viscometers. Unless shear effects are eliminated, the relation between $[\eta]$ and DP is not absolute. It should be noted that since polymer molecules are more extended in good solvents, errors from shear effects will be minimized in poor solvents.

In addition to providing a ready means of determining DP, the relation between $[\eta]$ and DP serves another useful purpose. For example, a comparison of the degrees of polymerization of various celluloses, amyloses, or amylopectins obtained by different workers gives no information concerning the reliability of the values, since the DP of a particular sample depends entirely on its history; the relations between $[\eta]$ and DP are, on the other hand, comparable.

Cellulose Trinitrate and Secondary Cellulose Acetate

The published values for K and α, both for cellulose nitrate and secondary cellulose acetate in acetone, have recently been critically surveyed.[3,14] For the nitrate, even after corrections for differences in nitrogen content were made, α was found to vary between 1·05 and 0·75 when the degrees of polymerization were determined by osmotic pressure measurements, and between 1·0 and 0·79 when they were determined by light-scattering and sedimentation methods. The ranges of the values of K were found to be $8{\cdot}75 \times 10^{-3}$–$44{\cdot}6 \times 10^{-3}$ and $5{\cdot}74 \times 10^{-3}$–$38{\cdot}5 \times 10^{-3}$, respectively. The often-vaunted superiority of light-scattering and sedimentation methods is obviously very much open to question. Among the relations that have been derived for the secondary acetate, based on measurements of osmotic pressure, α was found to vary from 1·0 to 0·67 and K from $4{\cdot}35 \times 10^{-3}$ to $36{\cdot}9 \times 10^{-3}$. Extreme values of K and α tend to compensate one another; a low value for α is generally associated with a high value for K. In particular, it was noted that some of the discrepancies were not real but had arisen through calculating values of K and α from data covering only a narrow range of DP.

In recent years the relations between $[\eta]$ and DP, for the principal derivatives of cellulose, have been the subject of intensive research



Curve 1: Cellulose trinitrate in *n*butyl acetate, 25°
Curve 2: Cellulose trinitrate in acetone, 25°

Figure 1. Relation between intrinsic viscosity and degree of polymerization

at the Shirley Institute. Secondary cellulose acetates (DP : 100–1,200) and cellulose trinitrate (DP : 100–1,800) that had been fractionated three or four times were used, and degrees of polymerization were determined osmotically.[3,14] A comparison of sedimentation (diffusion) and osmotic data for three nitrate fractions and seven acetate fractions[15] confirmed that the fractions were reasonably homogeneous and justified the relation of intrinsic viscosity to a number-average DP. The relations for trinitrates in *n*butyl acetate and acetone, and for secondary acetates in 80 per cent acetone, 20 per cent water (by volume) are shown in Figures 1 and 2 respectively. The equations of the lines are

cellulose trinitrate in *n*butyl acetate, 25° ... (1)

$$[\eta] = 0{\cdot}0141\ (\mathrm{DP})^{0{\cdot}969}$$

cellulose trinitrate in acetone, 25° ... ... (2)

$$[\eta] = 0{\cdot}0142\ (\mathrm{DP})^{0{\cdot}933}$$

sec-cellulose acetate in 80/20 acetone/water, 25°

$$[\eta] = 0{\cdot}0198\ (\mathrm{DP})^{0{\cdot}803}$$

It was also shown that for the secondary acetates in acetone at 25°

$$[\eta] = 0{\cdot}0244\ (\mathrm{DP})^{0{\cdot}760}$$

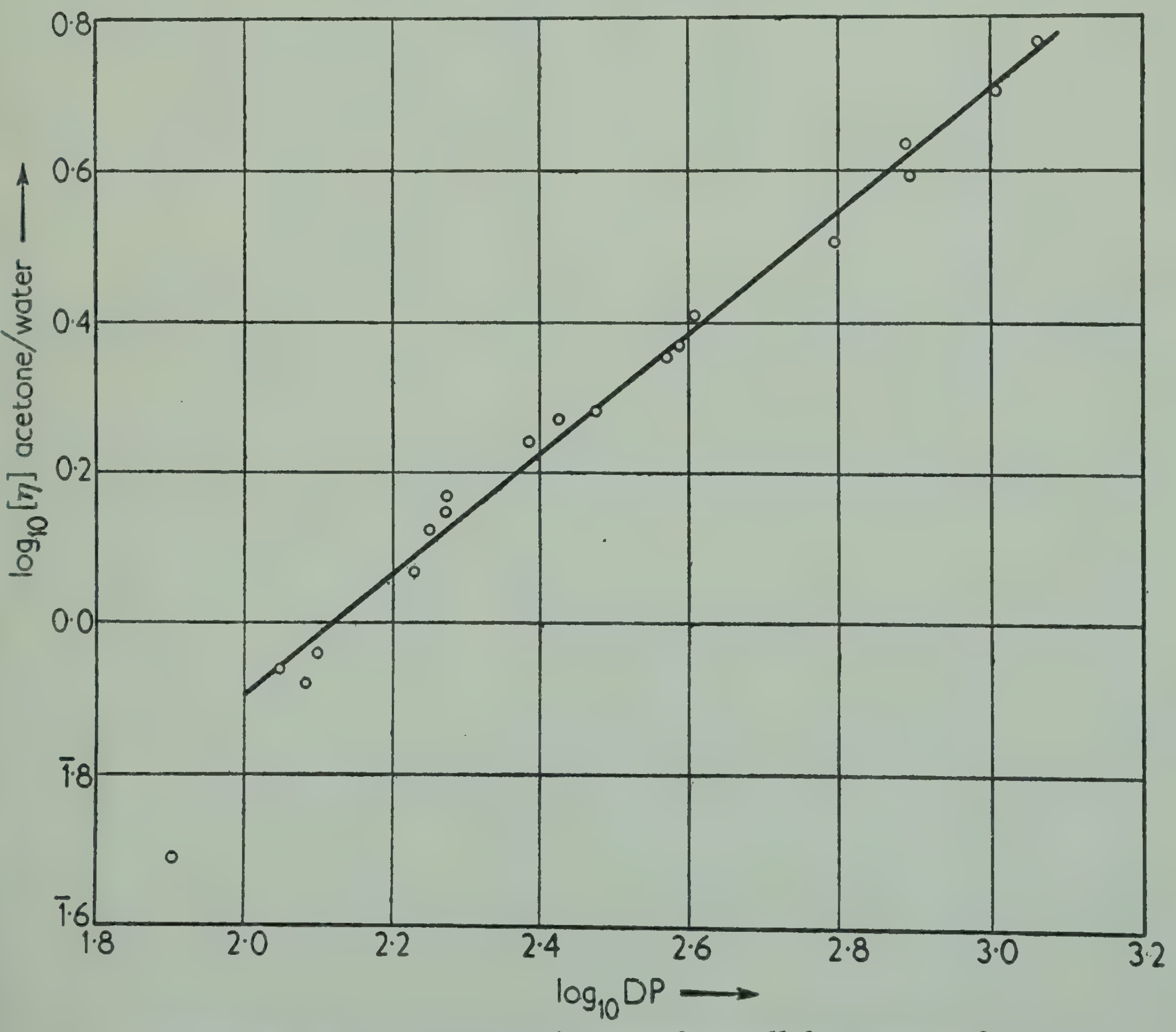


Figure 2. Relation between [η] *and DP for secondary cellulose acetate fractions in 80 per cent acetone, 20 per cent water, by volume, 25°*

The degrees of polymerization determined osmotically, and b measurements of sedimentation velocity and diffusion, are show in Table 2. It will be seen that the agreement between the tw methods is reasonably good. The discrepancies may stem from th heterogeneity of the fractions or from errors in either, or both methods used to determine the degrees of polymerization. There no absolute means of distinguishing between these possibilities, bu

Table 2: Comparison of the degrees of polymerization of cellulose nitrate an acetate fractions measured by osmotic and sedimentation (diffusion) methods

Fraction	Osmotic DP	Sedimentation (diffusion) DP	DP of nitrate calculated from column 6 and equation (1)	[η](dl/g) 80/20 v/v acetone–water	[η](dl/g) (14·14 per cent N) *n*butyl acetate
Nitrate	—	1,566	1,326	—	15·0
,,	—	771	791	—	9·08
,,	—	373	357	—	4·20
Acetate	408	470	(420)*	2·58	(4·92)*
,,	286	280	(282)	1·91	(3·43)
,,	249	200	(260)	1·74	(3·09)
,,	202	200	(208)	1·40	(2·49)
,,	176	200	—	1·17	—
,,	126	145	—	0·828	—
,,	80	83	—	0·485	—

* The figures in parentheses relate to the acetate fractions that were deacetylate and then nitrated.

it should be noted that the osmotic degrees of polymerizatio correlate better with the intrinsic viscosities than do the value derived from sedimentation (diffusion) data. Furthermore, sinc the average DP obtained from the latter is much closer to a viscosity average than is the number-average, it is unlikely that the poo correlation can be attributed to heterogeneity of the fractions.

Additional evidence for believing that the osmotic degrees o polymerization are reliable was obtained in two ways. Firstly b determining the degrees of polymerization of six secondary acetat fractions in four different solvents and in one solvent at two temperatures;[14] the results are given in Table 3. With the exception o the three fractions of lowest DP in pyridine, the degrees of polymerization in the different solvents are the same within experimental error. If the pyridine contained some water then th tendency of aqueous pyridine to swell the Cellophane membrane, thereby impairing its semipermeability, would explain the anomaly. Secondly, by comparing the osmotic degrees of polymerization of four acetate fractions with the values calculated from equation (1) and the intrinsic viscosities of the corresponding trinitrates, which were prepared by deacetylating the acetate fractions and nitrating the regenerated celluloses.[3] It may be seen from the results, which have been included in Table 2, that the agreement is excellent. The results also constitute proof that nitration without degradation is possible.

Table 3: Intrinsic viscosities and degrees of polymerization of cellulose acetate fractions in different solvents

Acetone/ water 25°	Ethyl lactate 25°	Pyridine 25°	Pyridine 60°	Methylene chloride/ ethanol 20°	Acetone 25°	Mean DP
397	407	415	413	420	399	408
291	298	279	283	278	—	286
257	243	255	254	238	—	249
193	189	214	216	199	—	202
172	170	184	185	171	—	176
123	120	135	136	118	—	126

Cellulose

The $[\eta]$–DP relation for cellulose in cuprammonium was arrived at by two independent methods.[16] The intrinsic viscosities of two homologous series of hydrocelluloses were determined in cuprammonium and the viscosity-average degrees of polymerization evaluated from the intrinsic viscosities of the corresponding trinitrates in acetone or *n*butyl acetate, and equations (1) and (2). The $[\eta]$–DP relation was found to be

$$[\eta] = 0{\cdot}0291\ (\mathrm{DP})^{0{\cdot}661}$$

The alternative approach, which is more direct, was to deacetylate secondary cellulose acetate fractions of known DP and to determine the intrinsic viscosities of the regenerated celluloses in cuprammonium. The relation between $[\eta]$ and DP was calculated to be

$$[\eta] = 0{\cdot}0319\ (\mathrm{DP})^{0{\cdot}657}$$

The two sets of experimental results are shown in Figures 3 and 4 respectively; the broken line in Figure 3 is the relation shown in Figure 4. The agreement is very close, the difference in DP

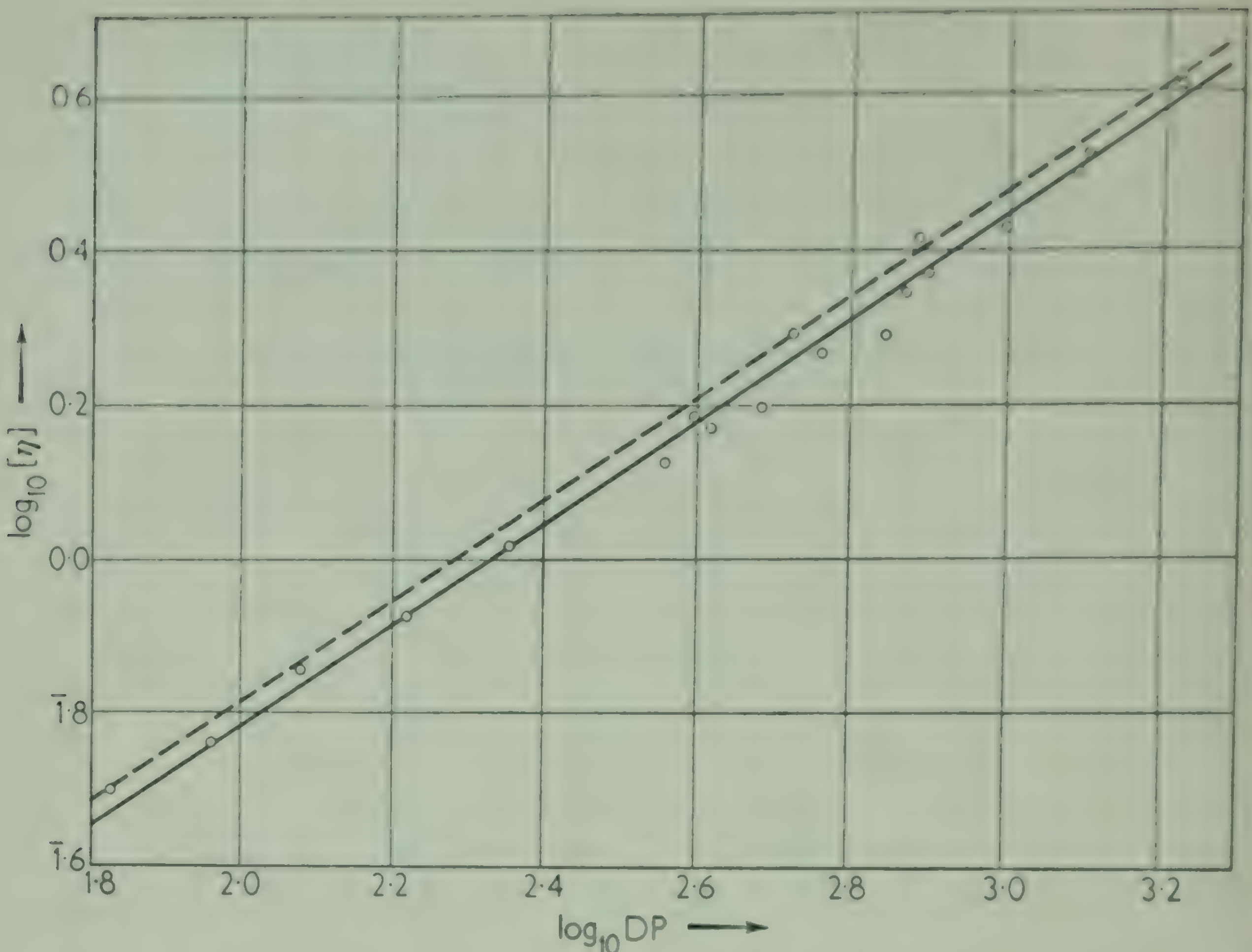


Figure 3. Relation between $[\eta]$ *and DP for hydrocelluloses in cuprammonium, 20°*

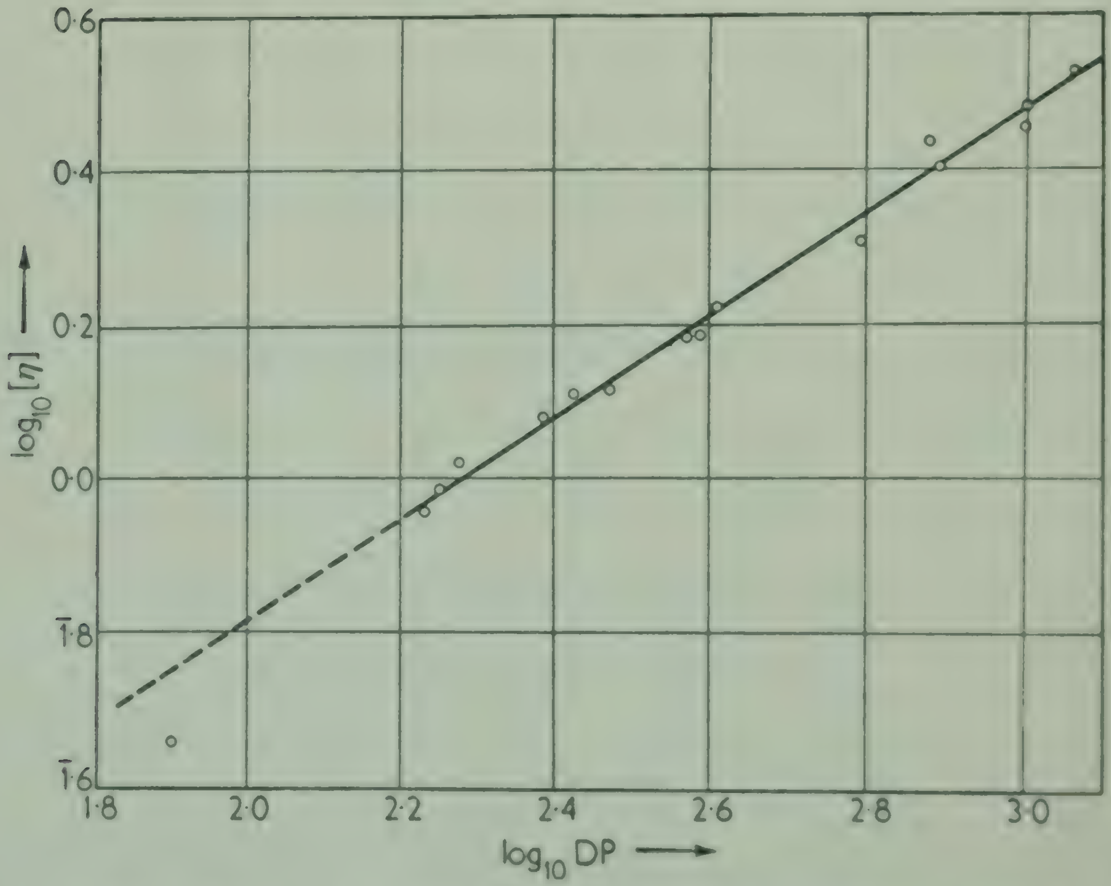


Figure 4. Relation between $[\eta]$ *and DP for saponified secondary acetate fractions*
cuprammonium, 20°

calculated from the two relations being about 10 per cent. The relations cannot be expected to agree exactly. The fractions are substantially molecularly homogeneous and the [η]–DP relation derived from them is therefore unequivocal. On the other hand the hydrocelluloses and their nitrates will have broad distributions of DP and, since α is appreciably different for cellulose in cuprammonium and cellulose trinitrate in acetone or *n*butyl acetate, the intrinsic viscosities of these two systems relate to a different parameter of the molecular length distribution, and are therefore not strictly comparable. However, it was shown that for two rayons, differing widely in DP distribution, the viscosity-average degree of polymerization when $\alpha = 1$ (the weight-average) and $\alpha = 0{\cdot}658$ differ by 7 per cent in one case and 11 per cent in the other. Since the difference is about 10 per cent for such dissimilar distributions it is reasonable to assume that the different viscosity-averages for the hydrocelluloses will differ by about the same amount. This difference of about 10 per cent operates in the right direction to account entirely for the discrepancy between the two [η]–DP relations obtained for cellulose in cuprammonium.

Cellulose Triacetate

The [η]–DP relation for cellulose triacetate has been evaluated by determining the intrinsic viscosities of two homologous series of

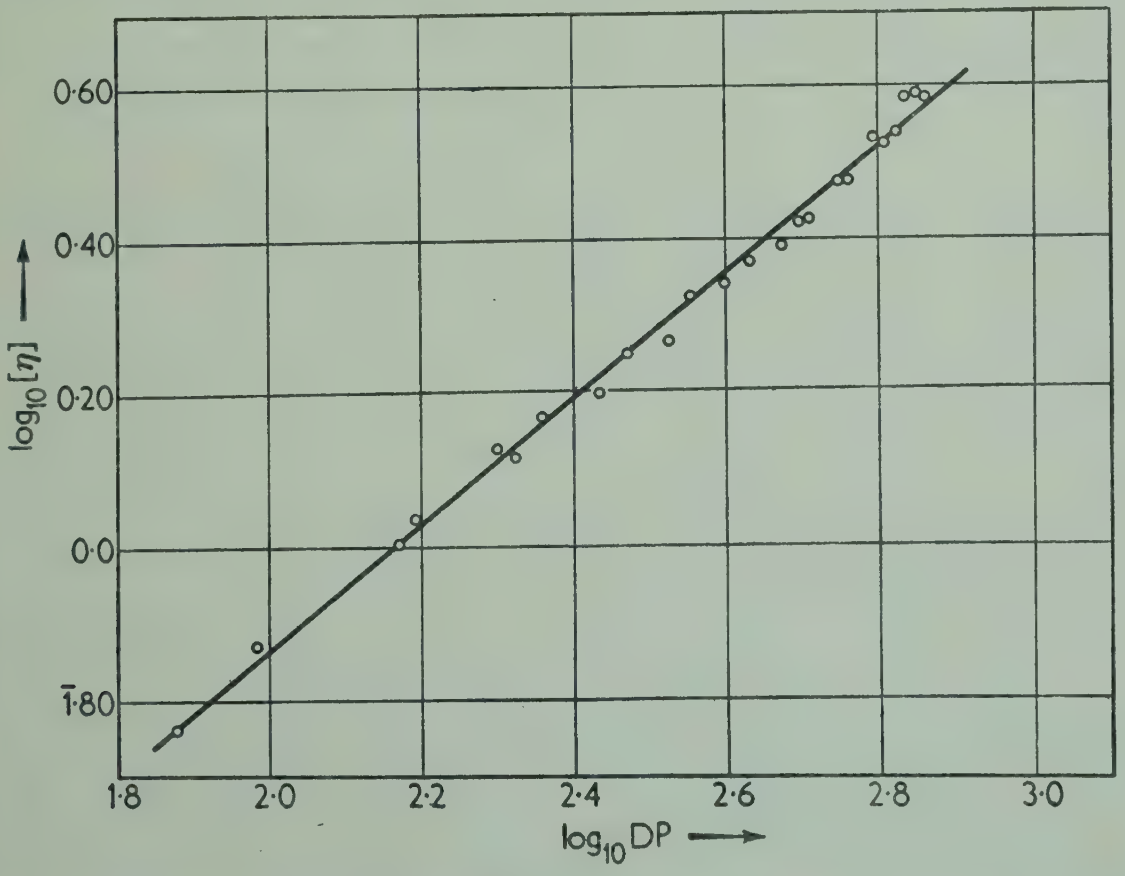


Figure 5. Relation between [η] and DP for cellulose triacetate fractions in 80 per cent methylene chloride, 20 per cent ethanol, by volume, 25°

triacetate fractions in 80 per cent methylene chloride, 20 per cer ethanol (by volume), and calculating their degrees of polymerizatio from cuprammonium intrinsic viscosities of deacetylated fractions.[1] The fractions were not refractionated, but it was assumed, on th basis of the above work, that they were sufficiently homogeneou to justify the correlation of slightly different viscosity-averages. Th relation obtained is shown in Figure 5, and the equation of the line

$$[\eta] = 0{\cdot}0156\ (\mathrm{DP})^{0{\cdot}834}$$

Flexibility of Molecules in Solution

If α is not equal to unity then equal increments of DP correspon to progressively smaller increments of intrinsic viscosity. Sinc intrinsic viscosity is a measure of the effective volume of the polyme molecules in solution, α must be some measure of the tendency c polymer molecules to coil in solution, and is therefore indirectly measure of chain flexibility.

From stereochemical considerations the value of α would b expected to increase as the degree of substitution increases, that i in the order cellulose, secondary cellulose acetate, cellulose trinitrat and cellulose triacetate; the last two being similar in this respect An examination of the values of α obtained shows that this i substantially the case, the exception being cellulose triacetate witl α appreciably below that of the trinitrate. It can only be conclude that 80 per cent methylene chloride, 20 per cent ethanol (by volume is a relatively poor solvent and promotes molecular coiling.

THE DEGREE OF POLYMERIZATION OF AMYLOSE AND AMYLOPECTI

The position regarding the degree of polymerization of amylose and amylopectins is more obscure than that of celluloses. All th worthwhile available knowledge can be summarized in the state ment that amyloses contain molecules with degrees of polymeriza tion up to a few thousand,[17] and amylopectins have weight-averag degrees of polymerization of the order of one million.[1] Informatio about these substances is lacking partly because there have bee relatively few investigations, but mainly because of considerabl experimental difficulties. Higginbotham[17] working with acetylate amyloses obtained different degrees of polymerization in differen solvents, and different [η]–DP relations for amyloses from tapioc sago, and maize, which he attributed to chain branching. Amylo pectins have such low intrinsic viscosities that relating intrinsi viscosity to degree of polymerization is precluded, and such hig molecular weights that osmotic pressure measurements are of doubtful validity.

It is obvious that before any real advance can be made in this field the accuracy of sedimentation and light-scattering methods will have to be considerably improved and standard samples of amyloses and amylopectins will have to be sent to different laboratories for confirmatory measurements to be made.

DEGREE OF POLYMERIZATION AND MECHANICAL PROPERTIES OF CELLULOSE FILAMENTS

The preponderance of work relating to the determination of the DP of cellulose and its derivatives can be mainly attributed to the interests of the rayon and plastic industries in the relation between DP and mechanical properties. Before discussing the results that have been obtained in this field it is interesting to see how the molecular structure of a rayon is related to its tensile strength.

The currently favoured structural arrangement of molecules in cellulose fibres is based on the so-called ‘ fringe micellar theory ’. According to this theory, crystalline regions (crystallites) are interrupted by amorphous regions characterized by a looser and more random packing of molecules that fail to achieve a crystalline lattice. As the name of the theory implies, the crystallites do not exist as distinct entities; molecules may pass through one or more crystalline regions, molecules may begin and end in crystalline or amorphous regions, and molecules may begin in amorphous regions and end in crystalline regions.

Hydrogen-bond formation in the amorphous regions is considered to result in a network structure that can be deformed under an applied stress. Small stresses are believed to result in the strain of secondary (mainly hydrogen bonds) and possibly some primary (covalent) valency bonds, and they are substantially reversible (elastic behaviour). Larger stresses are thought to deform the network structure and eventually to result in molecular rearrangements that involve rupture of hydrogen bonds, and the formation of new ones (irreversible extension). The additional force necessary to break the fibre has therefore to rupture covalent bonds in molecules spanning two or more crystalline regions; this force will be in excess of that required to overcome hydrogen-bond formation between molecules of the amorphous regions as they subsequently slide past one another. However, calculation has shown that, if the limit of strength were set by that of covalent bonds, a cellulose fibre could be many times stronger than is actually observed.[18] The upper limit is never reached because the breaking strength of an individual specimen is not an average property of the materi comprising it, but a property of its weakest part. Consequently, th uneven distribution of stresses within a fibre under tension will caus the most heavily loaded chains to rupture first, followed by the res a process analogous to tearing.

It can be appreciated on the ground of pure probability that, othe things being equal, the greater the average length of the molecule the greater the chance of their passing through two or more crysta lites; the greater therefore will be the number of molecules linkin the crystalline regions together and the greater the strength of th fibre. Since several crystallites can be linked either by one molecul or by a number of smaller molecules to form structures of equa strength, the tensile strength should cease to increase beyond certain chain-length.

Molecular length is not the only factor controlling the tensil strength of fibres; the degree of orientation of molecules an crystallites is also important. An increase in orientation results no only in an increase of the number of chains available for supportin a load, but also favours increased crystallinity with marked improve ment in the wet-strength of a fibre and decreased chemical reactivity In practice, tensile strength cannot be increased to the maximur possible by this means for the increase of strength is accompanie by a decrease of that in the transverse direction (cf. Cellophane elongation at break is also lowered and a compromise must b made. A too-highly oriented fibre is likely to have poor resistanc to bending and to split on twisting (cf. ramie); it should be note that cotton is safeguarded in this respect by the spiral constructio of the secondary cell-wall fibrillae.

From measurements of breaking strength, elongation to brea bursting strength, resistance to fold, and tearing strength of film cast from molecularly homogeneous fractions of cellulose nitrate,[19, secondary cellulose acetate,[21] and ethyl cellulose,[22] it has bee established that the mechanical properties increase rapidly up to DP of about 200 and thereafter remain constant. This resul however, can hardly be applicable to textile fibres or to plast goods manufactured by extension processes. Films cast on a surfa have a felt-like network of molecules,[23] which is quite unlike th molecular architecture of, for example, a rayon filament. Furthe more, long-chain molecules can be oriented more easily than sho ones and, if orientation leads to improved mechanical properti material of high DP is likely to appear of less consequence in film than in well-oriented products such as rayons and Cellophane.

A programme of work designed to fill the gap in the state of our knowledge concerning the influence of DP and molecular orientation has recently been completed at the Shirley Institute. Molecularly homogeneous fractions of secondary cellulose acetate have been spun by a wet-spinning technique[24] into model filaments, the filaments stretched to produce different degrees of orientation, and then saponified to produce cellulose filaments. The birefringence of the filaments was measured and used as an index of orientation; the load-elongation characteristics were determined with a Cambridge extensometer. The principal results of this work are shown in Figures 6-11. The ‘ effective stretch ’ referred to in the figures is not the amount of stretch applied mechanically but a value calculated from the denier (d_1) of the isotropic cellulose filament dried without tension, and the denier (d_2) of the stretched filament dried under the same conditions.

$$\text{Percentage effective stretch} = \left(\frac{d_1}{d_2} - 1\right) 100$$

This method of expressing the amount of stretch takes into account

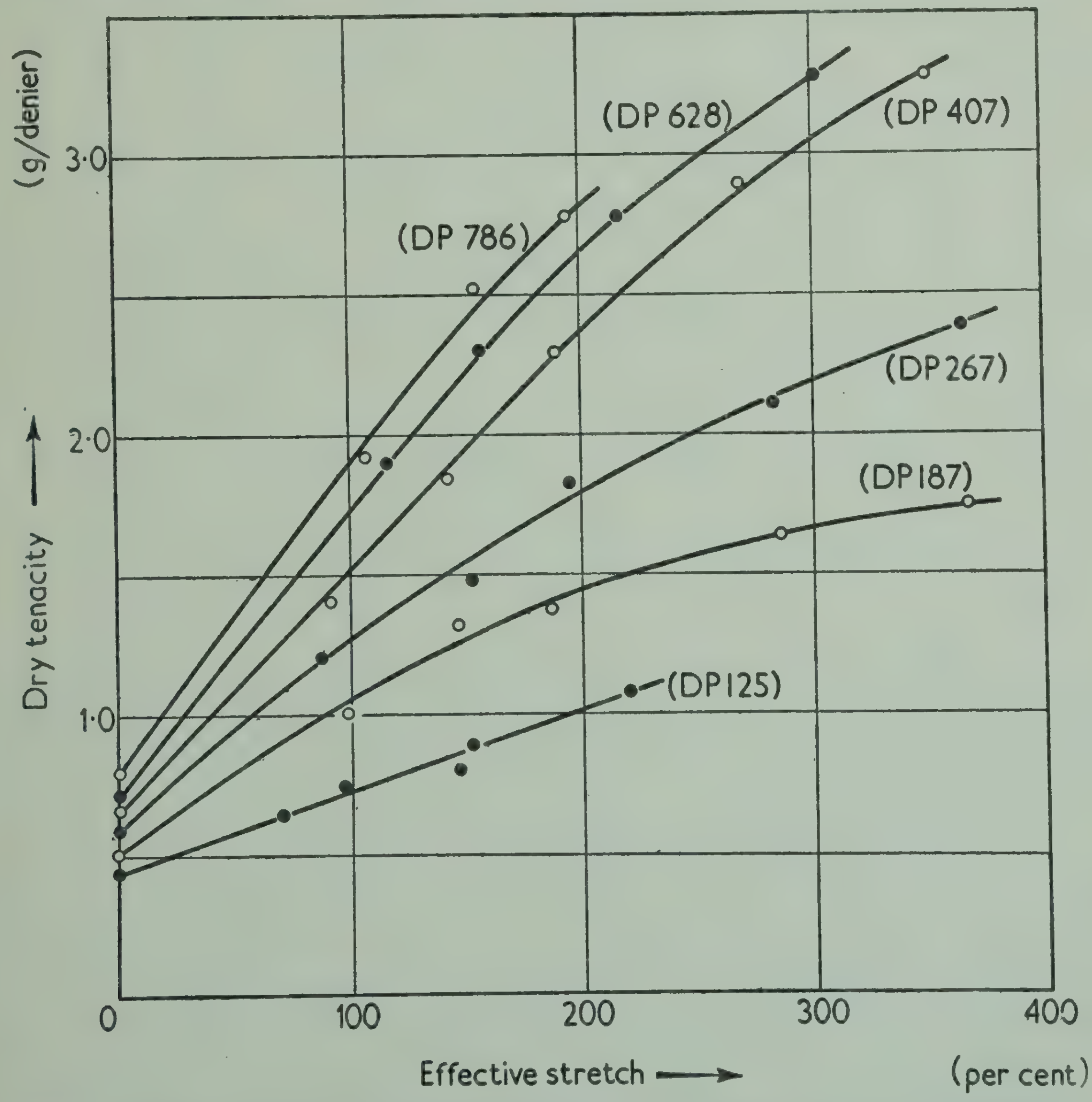


Figure 6. Effective stretch vs tenacity for filaments spun from fractions of different DP

the decrease in volume of the filament during the mechanica stretching process in boiling water, and during subsequen deacetylation (at constant length) and drying.

Figure 6 shows the relation between effective stretch and tenacit (breaking strength) in grammes per denier for filaments spun fro some of the fractions. This figure is included solely to illustrate th reliability of the experimental results, since the relations shown i subsequent figures are derived from data interpolated from simila graphs relating other combinations of the experimental result Figure 7 shows the relation between tenacity and DP for variou

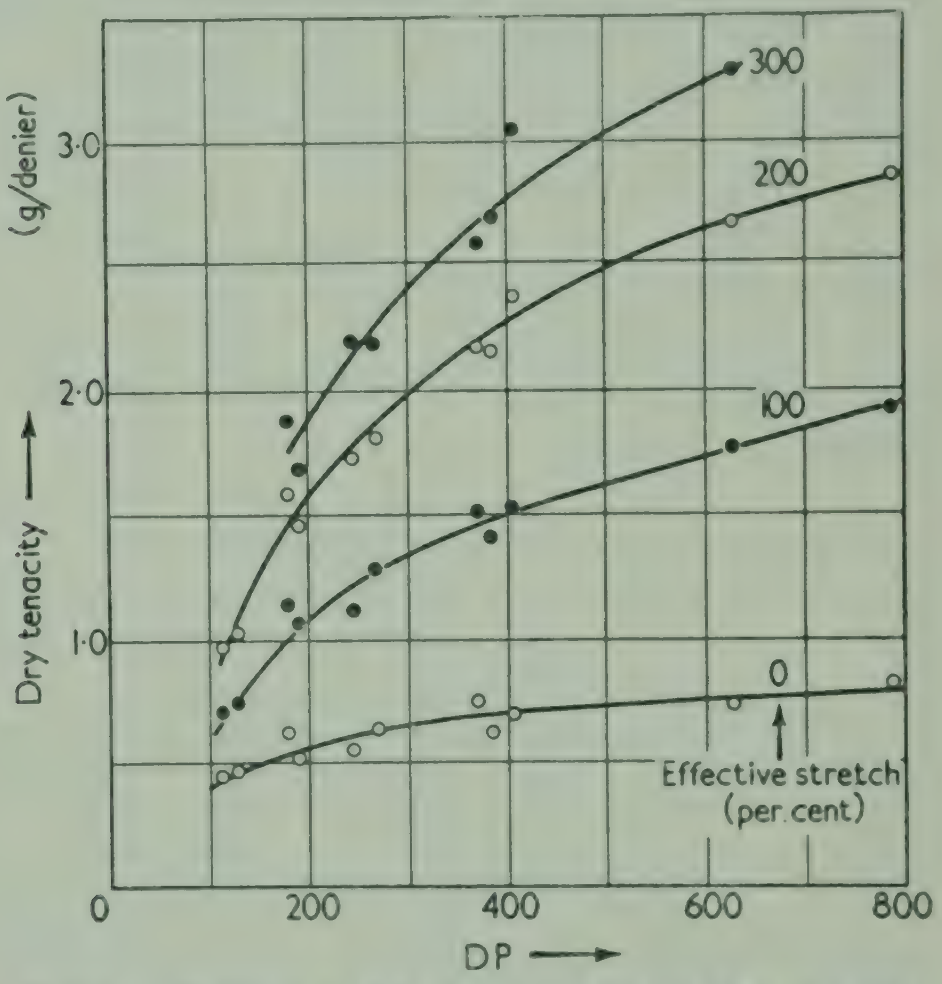


Figure 7. Tenacity vs DP for various levels of effective stretch

levels of effective stretch. It will be observed that for an isotrop filament (zero effective stretch) the tenacity ceases to increa when a DP of about 400 is reached. This is a similar conclusion that reached by working on films (see above). However, as t effective stretch increases, molecules of higher DP contribute mo and more to the tensile strength, and at the highest effective stret obtainable there is no limit in sight.

A graph relating birefringence to DP at different levels of effe tive stretch is shown in Figure 8. The continued rise of birefringen with increasing DP indicates that long molecules can be orient

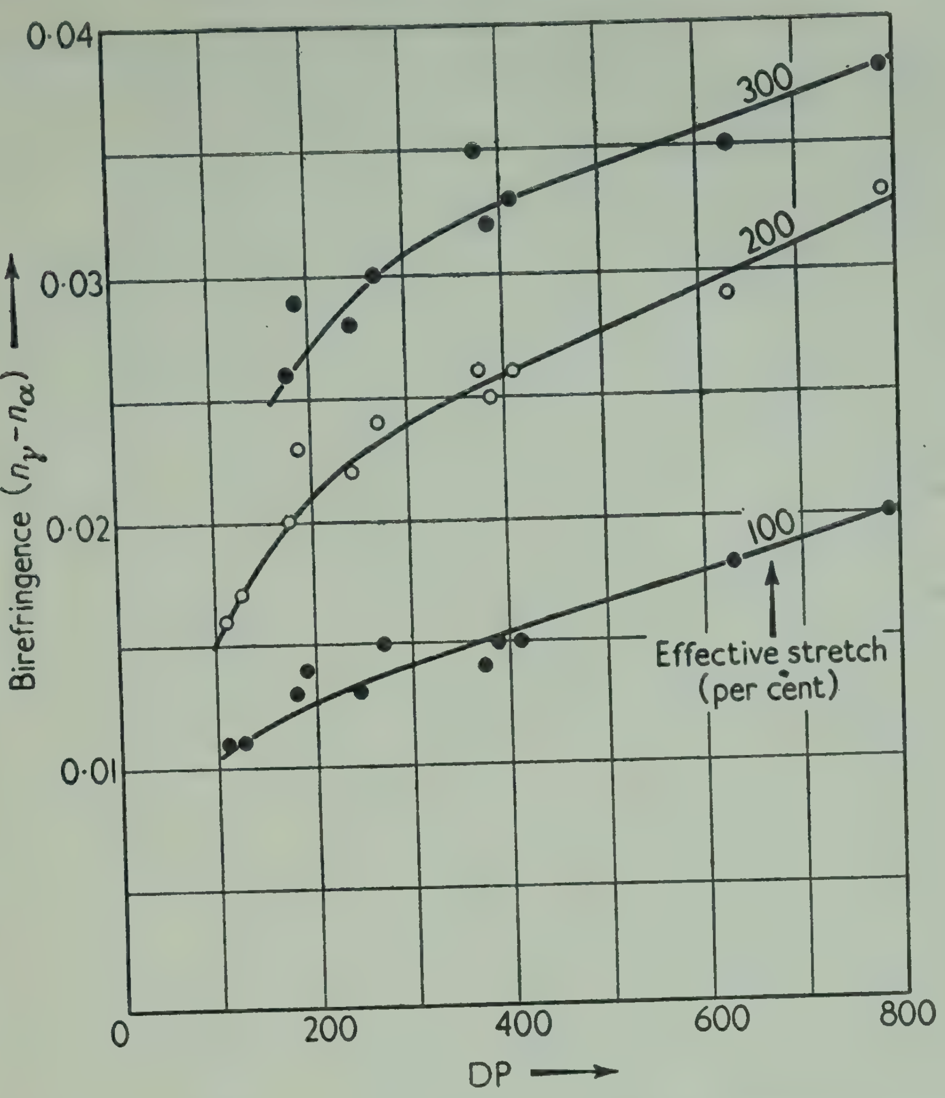


Figure 8. Birefringence vs DP for various levels of effective stretch

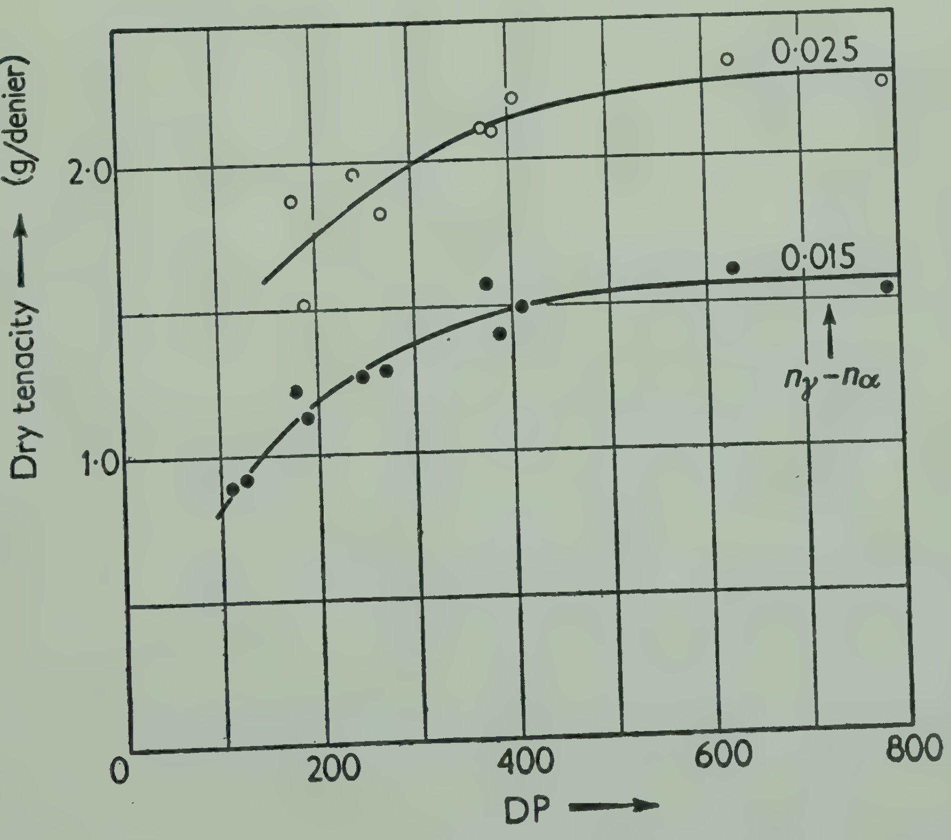


Figure 9. Tenacity vs DP at constant birefringence

more easily than short ones. It follows that the increase of tenacit with DP shown in Figure 7 must arise from the increasing chain length, and from the higher degree of orientation imparted to th longer molecules. This deduction is confirmed by the results show in Figure 9 where the tenacity is plotted as a function of DP a constant degree of orientation. Here it may be seen that the filamen strength ceases to increase beyond a DP of about four hundred.

Elongation at break is an important fibre property. Most rayon

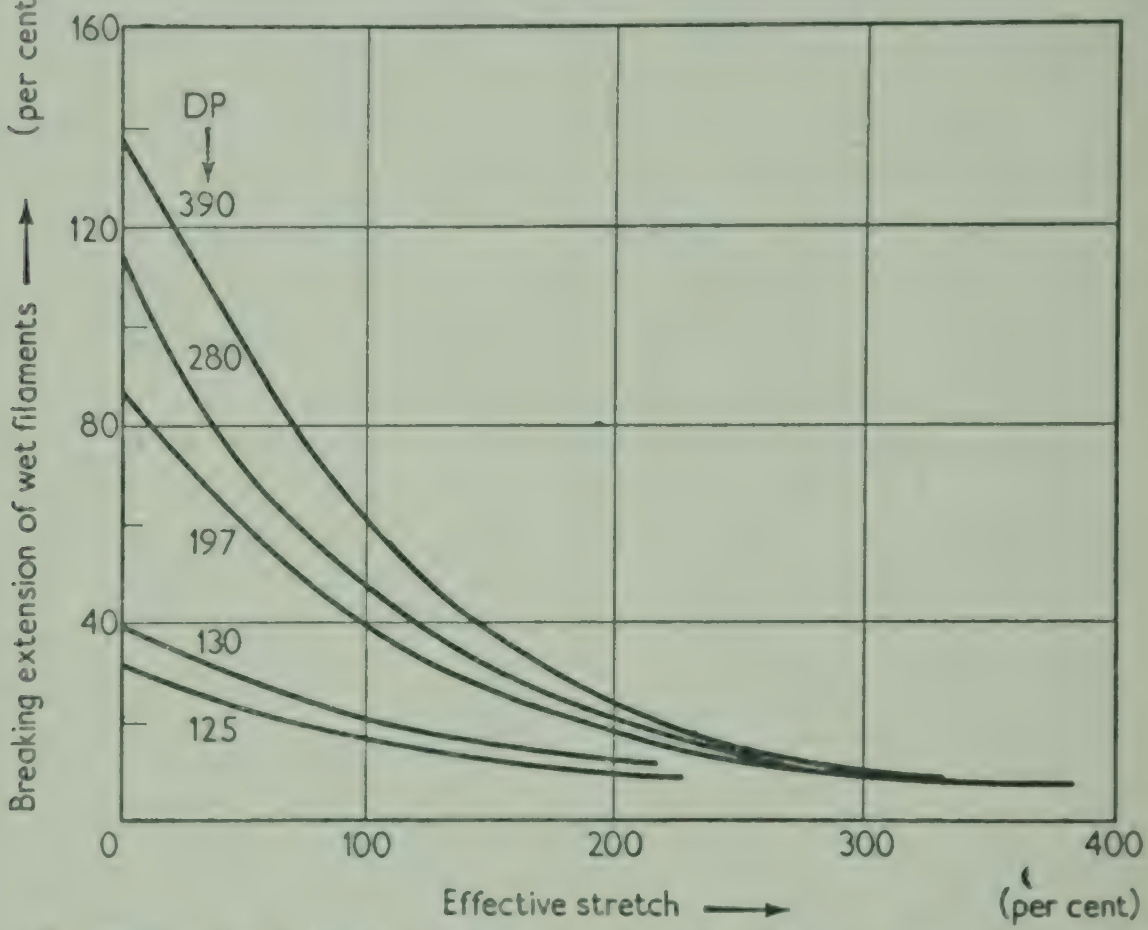


Figure 10. Extension at break vs effective stretch for filaments spun from fractions o different DP

for general textile purposes are spun under conditions that yield a fibre with a 20 per cent breaking extension. This requisite fixes th strength of the rayon, since the production of a more highly oriente rayon results in a decrease of the extension to break. By plottin the extension at break against the effective stretch (Figure 10) fo filaments spun from fractions with different degrees of polymerization it is seen that, provided the maximum effective stretch has no been reached, the breaking extension at constant effective stretc increases with increasing DP. In other words, for a given breakin extension a filament prepared from a fraction of high DP can b more highly oriented than one of relatively low DP, and a stronge fibre can therefore be produced.

The wet-strength of cotton is greater than the dry-strength

Unfortunately for the rayon manufacturer the reverse is true, and the wet-strength of rayon is much lower than the dry-strength. Bearing in mind that a breaking extension of about 20 per cent is required, it is interesting to plot the ratio of wet : dry tenacities of filaments prepared from fractions of different DP and having this breaking extension. The result is shown in Figure 11. There is no increase in the ratio beyond a DP of about 400, and it should be noted that the limiting value of 60 per cent is about the level generally accepted for ordinary viscose rayons.

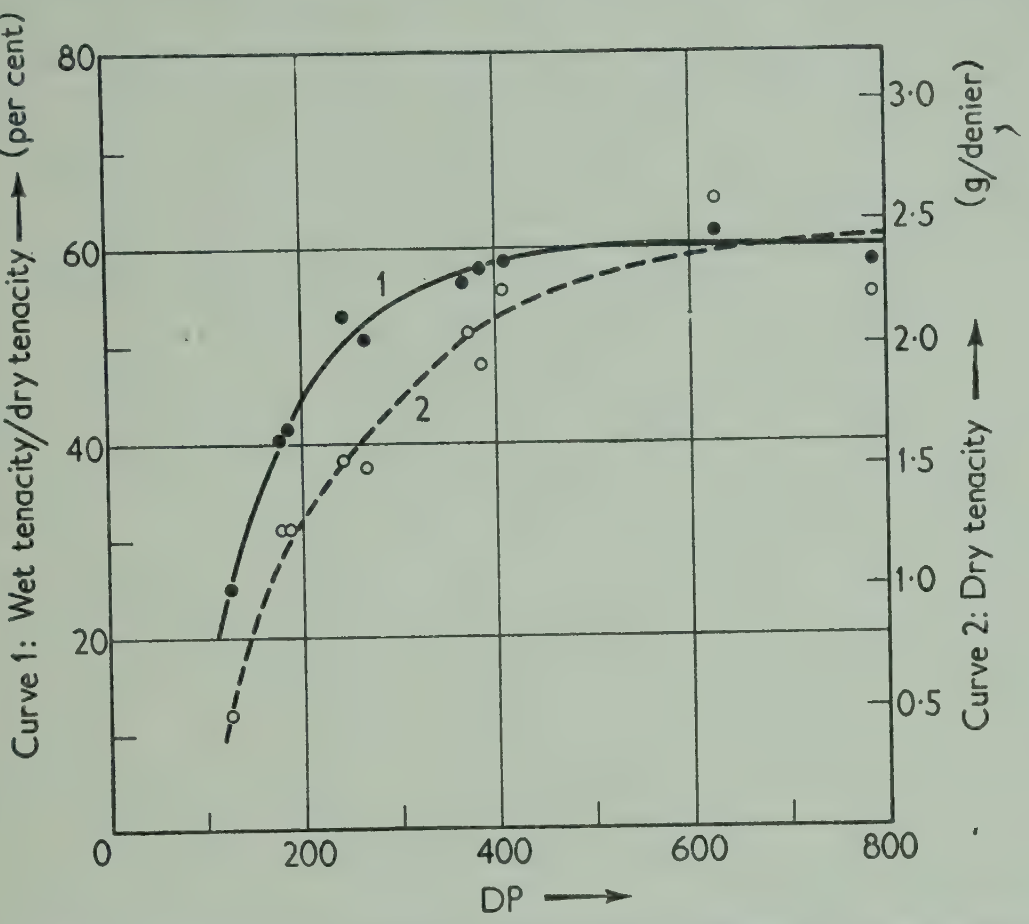


Figure 11. Ratios of wet to dry tenacities for filaments of different DP and with a breaking extension of 20 per cent

Fractions with degrees of polymerization up to 1,200 were available, but the structure of the concentrated solutions required by the spinning process resulted in unsatisfactory filaments if the DP was greater than about 800. Cumberbirch has also investigated mixtures of fractions, and re-spun rayons of known DP distribution.[25] He could find no evidence that the mechanical properties of mixtures of fractions are a function of their number-average DP as claimed by Sookne and Harris,[21] but confirmed their claim that the properties are additive on a weight fraction basis.

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11

PREPARATION, PROPERTIES, AND USES OF STARCH

STARCH, after cellulose, is perhaps the most widely distributed naturally occurring organic compound, being present in the tissues of many plants; in roots, or tubers of several; and making up the larger part of the solid content of seeds. Despite the great variety and wide distribution of the starch supply, only a limited number of products are used for the manufacture of starch on an industrial scale. These include maize (corn starch), potatoes (farina), manioc root (tapioca), wheat, and rice. More recently, new types of starches have been developed on a large scale from waxy maize, sorghum, and waxy sorghum.

PREPARATION

Regardless of the source of the starch, the general principles of preparation are similar in each industry, involving the wet milling to separate the constituents. The principles have remained very much the same for the past hundred years, but the application of these principles has undergone radical changes in the postwar period. Since maize (corn) from whatever source is by far the most important raw material on a tonnage basis, let us, therefore, take this as a typical raw material.

Figure 1 is a very much simplified flow sheet, indicating the main stages of manufacture. Maize of a suitable grade is first steeped in dilute sulphurous acid at approximately 50° for 48 hr in a steeping battery consisting of 8–10 tile-lined vessels, each capable of holding 50–70 tons of grain. The battery is filled in such a way as to enable counter-current leaching to be carried out continuously, the maize remaining stationary and the liquors being advanced by pumping.

Correct steeping is vital to the success of the subsequent milling stages, the main objects being to develop the correct degree of lactic acidity in order to soften the hull and partially solubilize the protein, to leach out the mineral salts present predominantly in the germ, and to avoid affecting the viscosity of the starch by over-steeping.

Steep liquor is drawn off the latest steep to be filled and the liquo is sent to triple-effect vacuum evaporators (for eventual use i penicillin or feeding-stuffs manufacture). By advancing liquor consecutively, the steeped corn in the earliest steep is drained read for processing. The germ is released by tearing the softened cor

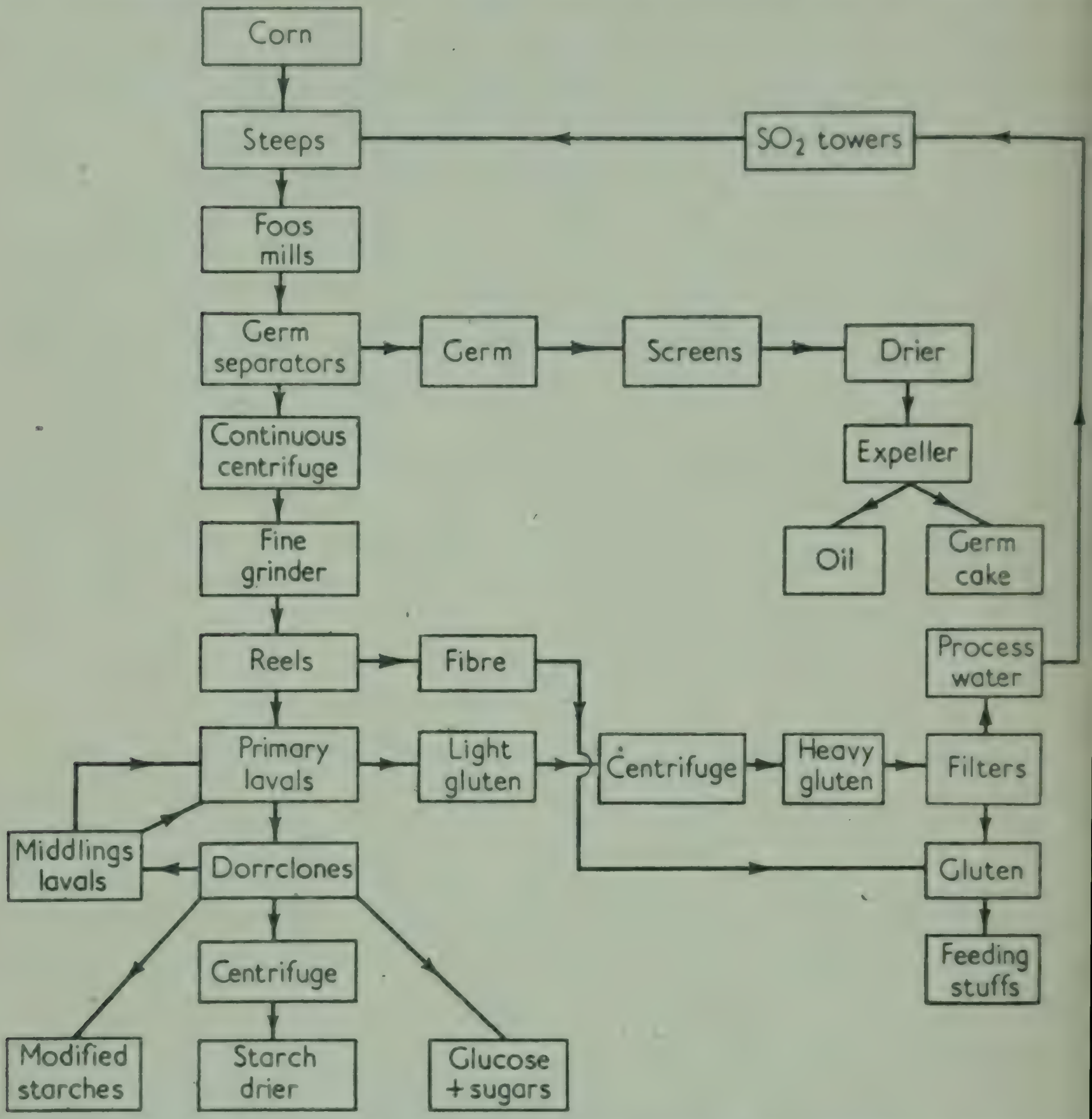

Corn
Steeps
SO₂ towers
Foos mills
Germ separators
Germ
Screens
Drier
Continuous centrifuge
Expeller
Oil
Germ cake
Fine grinder
Reels
Fibre
Process water
Primary lavals
Light gluten
Centrifuge
Heavy gluten
Filters
Middlings lavals
Dorrclones
Gluten
Centrifuge
Feeding stuffs
Modified starches
Starch drier
Glucose + sugars

Figure 1. Simplified flow sheet showing the principal stages in the separation of starch from corn

in Foos Mills, followed by a flotation process to recover the oil-bearing germs. Having removed the germ, the residue of starch, gluten (protein), and fibre is finally ground in the modern counterpart of the old Buhr stone mills to effect the maximum separation of starch from the gluten matrix. Fibre is next removed and washed by a series of counter-current revolving nylon-covered reels, or b continuous basket-type centrifuges, the fibre being subsequently dried and used for animal feeding-stuffs.

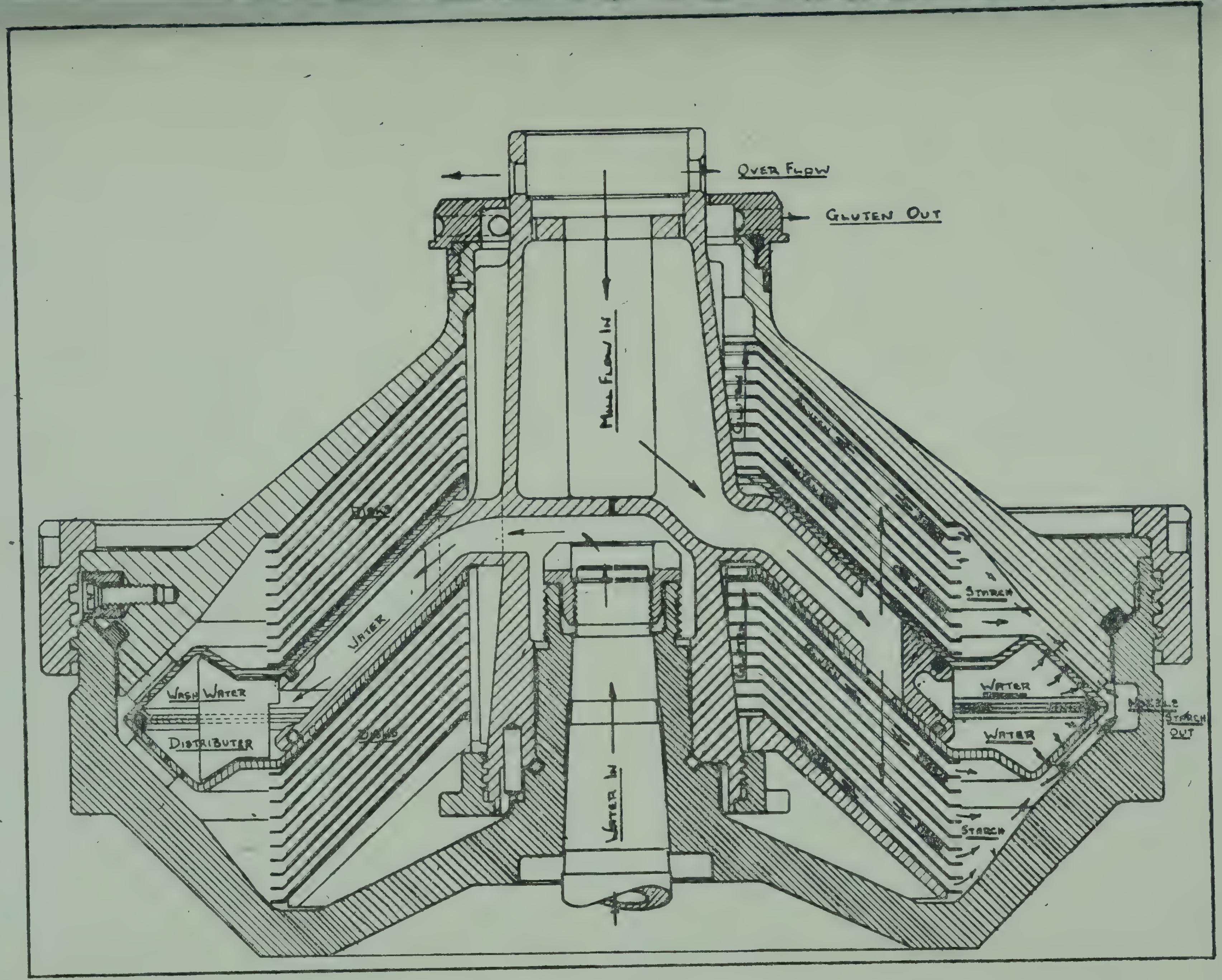

OVER FLOW
GLUTEN OUT
MILL FLOW IN
WATER
WASH WATER
DISTRIBUTER
WATER IN
STARCH
WATER
WATER
NOZZLE
STARCH OUT
STARCH

Figure 2. *Alfa Laval continuous separator used in the primary separation of starch and gluten*

The process of ' tabling ' to separate the starch from gluten is no longer practised in a modern plant, such an operation being more efficiently and economically performed by continuous centrifuges of the Alfa Laval or Merco type, followed by a second-stage purification in hydroclone installations (Dorrclones). Primary separation makes use of the well-known principle of the cream separators in which the mill flow liquor is fed continuously to a series of centrifugal machines, fitted with a wash-water device and yielding two streams —one, starch with a little gluten contamination, and the other, gluten with a little starch contamination.

Impure starch can then be passed through a second and third stage of the same type of machine to effect the necessary purification, or through a series of multi-stage Dorrclones, in which the washing is near perfection and an excellent low-protein product results. The principle of the Dorrclone is simply a fluid cyclone in which the starch liquor enters tangentially into the nylon clones. By controlling the rate of feeding and pressure, the necessary separation is achieved—starch leaving the underflow, and a gluten starch the overflow. This latter returns to the primary stage through a middlings concentrator.

To complete the picture, the light gluten is concentrated, filtered, and dried to be subsequently blended with the dry fibre to produce a high protein animal feed, whilst the water is recovered, passed to a sulphur dioxide tower to form steep acid, and so returned to the process. Fresh water enters the stream only at the Dorrclones for washing of the starch and leaves almost entirely as steepwater. The germ is expelled to produce oil for subsequent refining and germ cake, a very valuable component for feeding-stuffs. A large proportion of the maize starch so produced is converted directly without drying into glucose, and other solid sugars, and into the modified starches to which reference will be made later.

As an illustration of how rapid has been the advance in techniques in the postwar years, equipment which was the most modern until 2 years ago has already been superseded by still a newer and more efficient equipment.

PROPERTIES

Before discussing the many and varied uses of starches, it is necessary to consider the properties and behaviour in solution, and this in turn involves a knowledge of structure. By understanding the structure of starch, it should be possible to explain the behaviour in industrial applications and learn how to produce starches modified to suit specific applications.

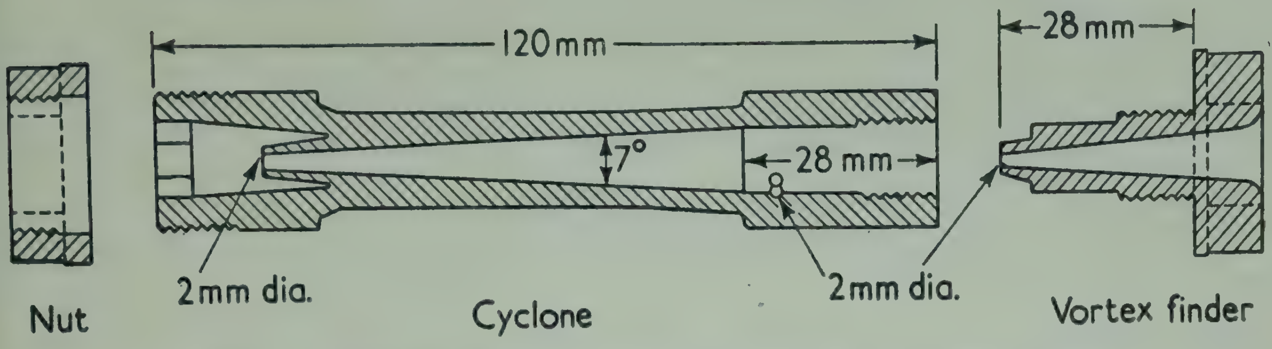

120 mm
7°
28 mm
28 mm
2 mm dia.
2 mm dia.
Nut
Cyclone
Vortex finder

Figure 3. Section of nylon Dorrclone, showing the tangential inlet

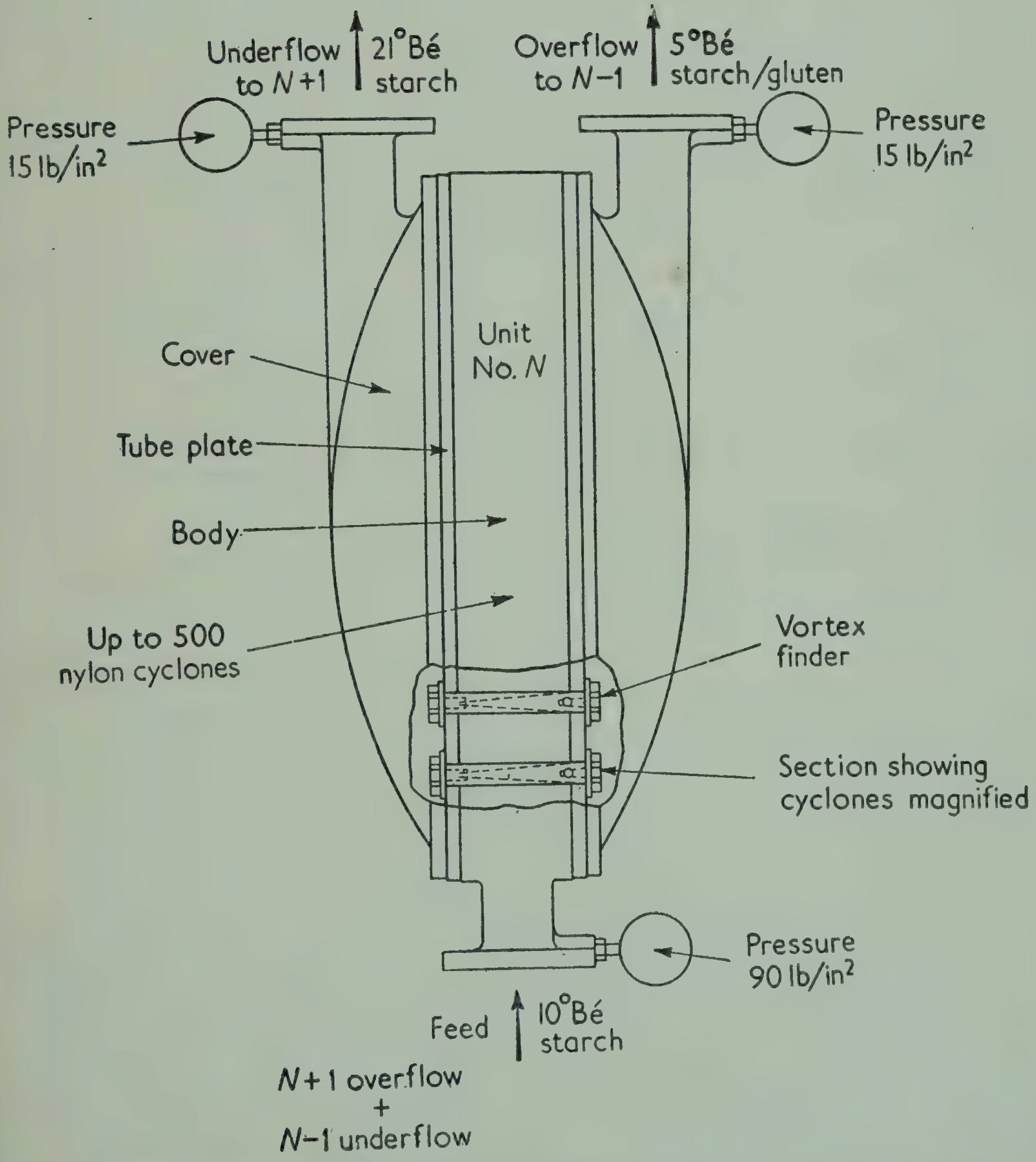

Underflow to N+1
21°Bé starch
Overflow to N−1
5°Bé starch/gluten
Pressure 15 lb/in²
Pressure 15 lb/in²
Unit No. N
Cover
Tube plate
Body
Up to 500 nylon cyclones
Vortex finder
Section showing cyclones magnified
Pressure 90 lb/in²
Feed
10°Bé starch
N+1 overflow + N−1 underflow

Figure 4. Dorrclone unit used for the final purification of starch. With 8 or 9 such units in series, true countercurrent washing of the underflow takes place—the underflow from the last stage being of required quality

Most of the research work prior to 1941 yielded conflicting evidence, although it was recognized by workers in the late nineteenth century that starch was heterogeneous. The main reasons for the inconsistencies are now attributed to the effect of impurities, such as fatty acids, residual protein, phosphates, and the labile nature of starch giving rise to hydrolytic degradation when attempting to form solutions for examination.

Probably the greatest advances stemmed from the work of Schoch[1] with the successful quantitative separation of linear and branched polymers by the use of selected solvents. Subsequent work has revealed that subfractionation is also possible and that starch, therefore, consists of several polymers. Undoubtedly, the best concise published account of the present understanding of the structure of starch is given by Greenwood in *Advances in Carbohydrate Chemistry*, Volume 11 (1956).

For convenience, it is now common practice to consider starch as a mixture of linear fractions called amylose and branched fractions called amylopectin. These fractions have quite distinct properties. Maize amylose, for example, readily associates and precipitates from dilute solutions; the phenomenon is called retrogradation. In more concentrated solutions, this confers remarkable gelling strength on maize starch, valuable for many applications, but also undesirable in others. It is this property which makes it necessary for freshly prepared solutions of starch to be used in volumetric analysis. Stable solutions can readily be made in the laboratory by dissolving a little dry starch in molten urea, grinding the mass when cool, and dissolving the product in cold water as required. The branch polymer is stable in solution and shows no tendency to retrograde.

It is extremely important to realize that the name amylose can be misleading if applied indiscriminately to the linear fractions of different starches. Thus, corn amylose is not the same substance as farina amylose, and both are different in behaviour from, say, tapioca amylose. Maize starch contains approximately 28 per cent amylose, farina 20 per cent, and tapioca 17 per cent, the remainder in each case being amylopectin. If these amyloses were identical in structure, then it should be possible to isolate, for example, the maize components by the solvent technique and then by reblending in different proportions produce a starch with identical properties to farina. This is just not true. However, there would be obvious advantages to be gained by separating the components if this were practical, since it would afford a method of controlling the solution properties to some degree. Selective precipitation by solvents has not so far proved to be a practical and economical proposition, but two alternative methods have succeeded.

The Dutch Process

Recently, farina starch has been successfully separated into linear and branch components by salting out.[2] The starch is cooked with a buffered magnesium sulphate solution at 160° for some 15 min, after which the solution is rapidly cooled to 70°. The farina amylose precipitates out completely whilst the amylopectin fraction is recovered from the mother liquor by subsequent cooling to 20°. Both products are now available commercially and uses have already been developed for the amylose based on the excellent film-forming properties.[3] One patent has been taken out for the application of amylose in the manufacture of skinless sausages, whilst the future developments may well be in the paper and textile fields.

Genetic Control

The second successful method makes use of a completely different approach and tackles the problem at its source, i.e. instead of attempting a separation of two naturally occurring polymers, it aims to develop strains of seeds which have a preponderance of one polymer to the virtual exclusion of the other.[4] This technique has been successfully developed in the United States by growing the so-called waxy starches (waxy maize and waxy sorghum) both of which yield starches which are essentially pure amylopectin. A more recent development has been a grain-yielding starch containing over 80 per cent amylose.

USES

Common Starches

At this stage we should remember that no one starch, such as maize, farina, sago, tapioca, rice, etc., has universal properties, and hence preferences in use developed for many years along empirical lines. For example, rice became the established laundry starch, primarily on the results of trial and error. Possibly this was influenced by availability, good colour, and the small granule size which allowed penetration of the cloth fibres in cold laundry work. Subsequent ironing in the wet state gelatinized the starch within the fibre mesh to give the desired superior finish. Today there are many industrial laundry starches based on wheat and maize, modified and blended to give excellent results, more economically than rice, many of the mixtures being developed for special applications.

Sago starch, on the other hand, is very much cheaper than rice, and though of variable quality and viscosity has proved very popular in the warp-sizing of cotton. Warp-sizing is the name given to the process whereby the threads are continuously passed through a bath of starch size and lubricant, in order to cover the thread with a protective film and at the same time improve the breaking strength. The threads passing out of the size-box pass over a drying drum and are wound on to beams ready for weaving. Without warp-sizing, continual breakage of the threads at the weaving stage would occur. After weaving, the size is removed by suitable washing, and possibly enzyme treatment, depending on the particular fibres being sized. The more recent use of the ' Shirley ' size-box regulator[5] has materially assisted in keeping sago well to the fore in the warp-sizing of cotton. Sago has much less retrogradation tendency than maize starch and this property has proved useful not only in cotton-sizing but also in the production of gravy and custard powders and certain types of liquid adhesive.

Farina retrogrades considerably less than maize and tends to give a stringy, cloudy paste, rather than the characteristic ' short ' paste of maize. On the other hand, farina, when cooked, is much more susceptible to a reduction of viscosity due to shearing, either by agitation or when the solution is being pumped. Farina is an excellent starch for making certain types of dextrines, and oxidized starches made from farina can yield very clear solutions. This latter property of well-oxidized farina starches is made use of in finishing certain textiles and in lace-dressing, where the brilliance of the finished colour must not be impaired.

Tapioca, in short supply for so many years, was considered as the best root-type starch for producing envelope and moistening gums by dextrinization. Due to the difficulty of supply and variable quality, attention has been focused on the newer waxy starches as suitable alternatives, and this particular field of application held for so long by tapioca is now being seriously challenged.

Pure maize starch without chemical modification has a very wide application in textiles, laundries, and paper and corrugated board manufacture, and an equally important field in the manufacture of foodstuffs of all kinds, including glucose manufacture. So many and diverse are the uses, that no attempt will be made to discuss each application, but rather to list a representative selection illustrating the properties required of the starch. Modified starches will be discussed later.

Pure maize starch, when correctly gelatinized by cooking in water, possesses very valuable colloidal properties, including high viscosity which (unlike farina) is not adversely affected by prolonged cooking, and the ability on cooling to develop a characteristic gel according to the concentration used. This property of gelling or thickening according to the concentration of the solution makes maize ideal as the basis for many foodstuffs, e.g. blancmange and similar desserts, custards, sauces, soups, ice cream, salad dressing, lemon curd, etc. In the non-food fields large quantities are consumed in manufacture of adhesive pastes, dry boiling pastes, dynamite and leather-dressing, in which starch paste is drummed into the flesh side of skins.

The strong gel formation is the property prized in textile-finishing to impart the required degree of stiffness and full handle to the cloth, and the same remarks apply to laundry-finishes where the addition of borax improves the gloss on ironing. The adhesion of pastes enables maize starch to act as a suitable binder in briquetting coal dust, asbestos fibres, and in the manufacture of insulating slabs from slag wool.

The dry properties which have proved valuable are particle size and shape, and the ability on compression to retain shape. Thus large quantities of starch are used in the manufacture of all types of rubber goods as a mould-release agent, and as a dusting powder to counteract the stickiness which rubber develops. This same property is made use of in production of toilet powders, and as a dusting powder in offset printing to prevent moist ink from smudging the sheet below.

A novel use of particle size is in the production of battery separators. This may be rubber or polyvinyl chloride compounded with starch and solvent. The hot soft mass is extruded to produce a continuous ribbon of the correct width, thickness, and rib design. After removing the solvent by drying, the strip is immersed in warm sulphuric acid baths to hydrolyse the starch to glucose, thus leaving the rubber or plastic with a microporous structure. Finger dressings are also being produced by the same principle to allow the plastic backing to breathe. When maize starch is compressed sufficiently it will retain impressions and this property is made use of in moulding starches. Impressions are made in trays containing compressed starch, so that casting of liquid gums, pastilles, marshmallows, etc., may be made in the individual moulds. After stoving and curing, the trays are inverted and the starch sifted from the sweets, dried to remove absorbed moisture and re-used for more impressions.

Bakeries use pure starch for a variety of purposes; baking powder utilizes free-flowing starch as a diluent for the bicarbonate and acid, and at the same time covers the chemicals with a protective coating against premature reaction, by absorbing moisture.

Due to the extremely low protein content, maize starch is blended with wheat flour to control the total protein content and thus modify the baking properties of the flour. This application is widely used in the manufacture of all types of biscuits to improve the texture and reduce the sugar and shortening required.

In spite of the variety of natural starches available their separate or combined properties will not meet the needs of modern applications and these deficiencies produced (and continue to produce) the demand for modified starches ranging from the early thin boiling, oxidized, and pre-gelatinized types to the latest adhesive starches, starch ethers, waxy starches, polyoses, and derivatized starches.

It is proposed to deal with each of these groups in turn giving examples of their principal uses in each case.

THIN BOILING STARCHES

One of the earliest modifications to raw starch was the production of a range of thin boiling starches. By treating a starch suspension with dilute acid at a suitable temperature, the acid soaks into the more open intermicellar areas of the granule, weakening bonds without affecting the form. When such a treated starch is subsequently neutralized to stop the reaction, washed, and dried, its appearance is the same as raw starch. When gelatinized in water, however, the weakened bonds break and give pastes with reduced viscosity. The linear fraction is only slightly affected (depending on the temperature, time of contact, and acidity) and still exhibits retrogradation tendencies according to the degree of scission. In some cases, the retrogradation is increased slightly showing that partial breakdown of the linear fraction has occurred and that the average chain-length is nearer the critical one for maximum association.

These starches are valuable when it is necessary to have a solution of higher solid content than can normally be obtained with unmodified starch and still retain the valuable retrogradation properties. Well-known examples are textile finishing in which the required degree of stiffness is of the same order as raw starch, but a heavier loading of the fibre is required, and collar starching utilizing the same principle where particularly heavy sizing is required.

Thin boiling starches of various fluidities are used in the manufacture of gums and jelly confections (Turkish Delight is a good example) where a high concentration of solids is required and the retrogradation property is of the utmost importance. In certain applications of this type of starch, similar results can be obtained by using a blend of corn starch and white dextrine.

Thin boiling starches have been found to have the required viscosity and nutrient characteristics in the production of Aureomycin.

WHITE DEXTRINES

If we attempt to thin the starch by the above process to a still greater degree, then an excessive amount of starch would be solubilized and lost during the washing process. To avoid this the thinner starches are made by heating dry starch at a controlled pH to form white dextrines. The degree of thinning and solubility will depend on the time, temperature, and pH. White dextrines will, therefore, have similar properties to thin boiling starches but with still lower viscosities.

Whilst textile-finishing consumes large quantities of white dextrines, another large application is in the manufacture of plaster board for the building industry. In the very early days of gypsum board manufacture, hand moulding was resorted to. The boards were dried in tunnel kilns over periods between 20 and 24 hr. Later, the method of manufacture was mechanized and although such methods were basically the same as those employed today, drying temperatures were much lower and drying times much longer. With the introduction of starch into the process, such starch being first mixed with the dry gypsum, much higher drying temperatures became possible with greatly reduced drying-time cycles.

The bonding of the paper board liners to the gypsum core may be described as being of a mechanical nature. The starch does not function as an adhesive, i.e. by gluing the paper board to the gypsum core. When the gypsum-water slurry sets (recrystallization of the hemihydrate into the dihydrate) long thin needle-like crystals occur. Since some of the water in the mix penetrates into the interstices of the paper before the recrystallization occurs, many of these fine crystals grow into the paper, locking with the fibres and forming a bond. The principal function of the starch, having been firstly blended into the dry gypsum, is to migrate to the core face during the early part of the drying operation and to form a protective barrier to the recrystallized gypsum, which is locked into the paper board. Without this protection, the crystals would be calcined in the subsequent drying, with complete loss of bond. For this process, the starch has to be carefully selected; it must have the property of readily migrating to the core face, and have the ability of holding water at the interface, in order to prevent calcination of the bonding crystals. The average consumption of starch is 8–10 lb/1,000 ft^2 o board.

YELLOW DEXTRINES

By treating dry starch with suitable acids, such as hydrochloric o chloracetic acid, to a higher temperature and for a longer time tha for white dextrines, the starch first undergoes a preliminar hydrolysis to small fragments corresponding to white dextrines. A the temperature is raised and moisture eliminated, the fragment appear to re-combine to give a type of branched structure. Th viscosity may rise during the re-polymerization, indicating a appreciable increase in molecular size. Whilst the precise mechanis is not understood, the net effect is to reduce the amount an influence of the linear portion. The degree of re-polymerizatio is very important since this will determine the property of th solution. With very little linear portion, the solution of dextrin would be stable and be satisfactory for production of gumme paper, since it would yield a clear glossy film when dry, i.e. it woul be a satisfactory re-moistening gum, e.g. for envelope manufacture However, for certain applications, the property of retrogradatio or ' set back ' is still required, and the degree has to be controlle by the conditions of cooking. In adhesives manufacture, this i probably the most important and most difficult property t reproduce. In carpet backsizing, the solution of yellow dextrine i used hot during application but must subsequently develop desirable degree of retrogradation on cooling and drying to give th hessian backing the correct handle and leathery feel and bind th tufts to the hessian to produce the desired tuft anchorage.

A large consumer industry for yellow dextrine is that of th foundry, where sand is bonded with dextrine and oil to produc cores with the required degree of ' green strength ' to enable th cores to be handled before baking and the necessary ' dry strength after baking to withstand the erosion and temperature when castin molten metal (see below, p. 301).

OXIDIZED STARCHES

By treating a starch suspension with sodium hypochlorite solutio in the presence of caustic soda, a number of carboxyl groups can introduced. Only a very small number (1 in 20 glucose residues 1 in 75 glucose residues) are required to distort the linear polym and prevent or reduce the tendency to associate. At the same ti the presence of hydroxyl ions degrades the starch, and since t degree of retrogradation depends upon the chain length, this too reduces the effect. The oxidized starches, with less tendency to retrogradation, have correspondingly improved film-forming characteristics. One such grade found ready acceptance as an excellent warp-size for cotton. The more highly oxidized starches have become indispensible to the paper-coating industry. Machine-coating by one (the Massey) process utilizes a coating slip made from starch and clay usually containing 55 per cent solids of which 10 per cent is modified starch. Coated papers produced by this method are in the lower price range compared with air-knife or brush-coated papers. The coating machine forms an integral part of the paper-making machine, such a unit being situated towards the end of the drying cylinders. The coating unit consists of a series of rolls, usually rubber covered, and set horizontally. The clay slip is picked up by the first roll, transferred to the others, and hence on to both sides of the paper.

One of the most recent developments in sizing paper is that of size-press sizing, using oxidized starches without clay. This has developed from the enormously increased printing speeds. Previously printing machines of 2,000 impressions per hour were considered very fast but today 10,000 impressions is common, and for one Swedish machine 18,000–20,000 impressions per hour are claimed. It is essential, therefore, to tie down surface fibres much more severely, otherwise the high speeds would be nullified by having to stop and clean fluff off the rollers.

A modern size-press is fitted into the paper-making machine, approximately two-thirds along the length of the drying cylinders (usually 6–8 cylinders are allowed after the size-press) and consists of two rolls running lengthwise across the machine. Early size-presses had the two rolls vertical, the lower one dipping into the starch solution and coating one side. The other side of the paper was coated by a spray pipe servicing the top side where it forms a constant pool overflowing to the trough below. Later developments have utilized horizontal rolls with the web of paper passing down through the nip, a spray of size being directed on each side of the paper to give more even distribution. Size solutions are made from oxidized starches (due to their filming property and availability in a range of suitable viscosities) at a concentration of 4–10 per cent and applied at approximately 50°. Pick-up varies from 1–5 per cent according to concentration of size, speed of machine pressure, of nip rolls, and quality of paper.

A process as yet in its infancy is size-press coating using starch ethers with a little clay. This process will produce special papers with a surface somewhat similar to normal clay-coated, but in a more economical range, since the amount applied will be considerably less.

STARCH ETHERS

If, on the other hand, retrogradation tendencies have to be reduced without loss of viscosity, this can be accomplished by treating the starch suspension with ethylene oxide and thus introducing hydroxethyl groups. A low proportion of derivative groups, perhaps one on every tenth or twentieth glucose units, is enough to interfere with the association of amylose. These products are the starch ethers. Not only is retrogradation arrested, but the starch forms a beautiful film. This property has been extensively used in the warp-sizing of spun rayon, for which the ease with which the size film is re-solubilized is of considerable importance. Spun rayon, on account of its low tensile strength when wet, must be handled with the greatest care. It is for this reason that starches having a readily soluble film are required for satisfactory sizing. The improvement in weaving performance is excellent, but varying reports have been received on the desizing properties. Recent modifications have produced a starch ether with remarkably good desizing properties, whilst still retaining the excellent weaving properties. Desizing is normally carried out at 80° for 15 min, followed by a second scour at the same temperature, but with 0·5 per cent soda ash added.

Similarly, satisfactory results have been achieved in warp-sizing Terylene–cotton blends, cellulose triacetate–viscose blends, and spun nylon. The formulations are based on these starch ethers blended with polyvinyl alcohol and spermaceti wax, with concentrations depending on the particular application.

In textile finishing, the non-congealing characteristics make these starch ethers ideal for blending with melamine- and urea-formaldehyde resins to impart crease resistance, dimensional stability, and a pleasant feel. As a guide, 0·75 lb/gal of starch with 25–50 per cent of resin based on its dry weight of starch forms a basic mixture. It is essential, in view of the resin content, to cook the starch with half the water for 30 min at 93° and then, when completely gelatinized, add the remaining cold water to reduce the temperature to 50° before adding the resin.

PRE-GELATINIZED STARCHES

A large demand for pre-gelatinized starches exists where cooking facilities are either not available or not convenient. For these applications, starch is slurried in cold water and fed to steam-heated rolls. Starch slurry rapidly gelatinizes and forms a dough roll which is automatically transferred to the main drum by one or more doctor rolls, thus producing a thin film of dried starch which is continuously removed by doctor knives as the drum rotates. Under the correct conditions of manufacture, the film leaves the drum in a broad continuous ribbon several feet wide, rather like rice-paper in appearance. When ground in a suitable mill, the product is stable and can be reconstituted quickly with water as required. The principal users of this type of product are the paper and foundry industries, whilst smaller quantities of pre-gelatinized modified starches form the basis of many cold water pastes including wall-paper-paste powder, in oil-well drilling, and in the production of certain types of instant dessert.

Paper and Corrugated Board

In the manufacture of paper, the pre-gelatinized starch is added in dry form directly to the pulp in the beaters or hydro-pulpers. Its function is to assist in the dispersion of fibres and of resin precipitated by alum. The starch, therefore, acts as an adjunct to the normal resin size and does not replace it. An addition of from 1 to 3 per cent on the dry weight of finish improves the bursting strength (Mullen factor) while at the same time imparting an improved handle, rattle, and surface.

From the inception of corrugated-board production on a commercial scale, and for many subsequent years, sodium silicate was the principal adhesive used in this industry for the bonding of the fluted medium to the liners. Starch-based adhesives were found to be unsuited to this purpose on account of their relatively low speeds of setting. This situation continued until some twenty-five years ago when the method of gelatinization *in situ* was perfected, the method being now generally known as the Stein Hall process. This process, which gained in popularity in the United States during the years immediately prior to 1939, has, during the past few years, found favour in Great Britain. In the United States, it is claimed that starch adhesive is now used for more than 80 per cent of the total board production. In this country, the majority of the large modern plants now employ starch as the adhesive, whilst others are gradually changing from sodium silicate. In this gelatinization *in situ* process, the adhesive consists of ungelatinized starch suspended in an aqueous solution of a gelatinized starch, having sufficient viscosity to keep the raw starch in suspension and yet to permit of the composition being properly ‘ picked up ’ and transferred on the applicator rolls on the machine. The first application in Britain used a blend of pre-gelatinized and raw starches in the proportions 1 : 6, respectively, in aqueous solution of borax and caustic soda; but at present the two-tank system is universally adopted. The tanks are mounted one above the other, being referred to as top and bottom tanks. In the normal Stein Hall process,[6] water, starch, and caustic soda are well agitated and heated to 65° for 15–20 min. The cooked starch is then diluted with a further quantity of water before being dropped into the bottom tank containing a borax solution in which is suspended the raw maize starch. The function of the caustic soda is to depress the gelatinization temperature of the suspended starch, thus giving far more efficient gelatinization at the appropriate point on the machine, this in turn permitting of faster running speeds. Borax is added to increase the viscosity developed when the raw starch is gelatinized, and as an effective buffer for the sodium hydroxide.

Corrugated board is made by a continuous two-step operation. It consists of corrugating paper by means of steam-heated fluted rolls applying adhesive to the tips of the corrugations on one side, bringing a paper liner in contact with the adhesive coated tips, and forming the bond with heat and pressure. This first step in the operation forms what is known as ' single-faced corrugated board ' and consists of a corrugated strip of paper, bonded to a smooth-surfaced liner. This phase of the fabrication is carried out on a machine known as the ' Corrugator ' or ' Single Facer '. The second step of the operation consists of applying an adhesive to the tips of the exposed corrugated surface, bringing a liner in contact with it and forming the bond with the assistance of heat and just sufficient pressure to hold the paper in contact without deforming the corrugations. This second phase is performed on a machine known as the ' Double Backer '.

Starch adhesive may be reinforced with various synthetic resins for the purpose of producing weather-resistant board. Within the past year an improved formulation has been developed, making use of the well-known effect of borax on cooked starch, from the standpoint of improving both weight and final adhesive bond. Whilst conventional Stein Hall formulations used this principle, the mixtures are so constituted that the reaction between borax and starch takes place after the adhesive is transferred to the board and heat is applied. In the new formulation, the reaction is effected in the cooked carrier itself, which then becomes considerably more than a suspending agent for the major uncooked part of the adhesive Due to the higher viscosities developed in the presence of borax more water is required. The technique is to cook the starch, borax, and caustic soda in the top tank, drop the mixture into the bottom tank containing water, and then use the top tank again to produce the cold raw-starch slurry, which when added to the bottom tank gives the prepared adhesive, thus reversing the normal order of addition.

Foundry Trades

The foundry trades consume pre-gelatinized starches on a very large scale. For more than thirty years, starches have figured in the treatment of foundry sands, largely to the exclusion of clays and other ingredients. During this period, this practice has developed with great rapidity, and for many years starches have found ready acceptance as an economic and easily controlled means of imparting the required degree of plasticity to these sands.

The two general types of sand considered are core sands and moulding sands.

Core sands. The great majority of foundries employ, for the production of cores, what is referred to as ‘ oil sand practice ’. In such practice starch is used to impart plasticity, with the necessary ‘ green strength ’ to enable the cores to be removed from the core boxes and transported to the drying stoves, with complete retention of shape. An oxidizing oil or resin is also added to impart the required degree of strength and hardness after stoving to enable the core to be fitted into the mould and to withstand the impact of the molten metal, until the metal begins to ‘ freeze ’, when the core should collapse. The careful adjustment of the ratio of starch and oil to silica permits the above requirements to be attained. The average percentage additions are: 1–2 per cent of starch and 1 per cent of oil, based on the weight of sand. The core is baked at 200°.

In the preparation of core sands, water plays an important role. Many foundries have facilities for pre-drying the silica sand, while others are able only to dry a portion of such sand, and many are obliged to resort to the use of wet sand as received at the foundry. In the development, therefore, of suitable starches, the above had to be borne in mind.

Two general types of starch were developed to meet such conditions. A pre-gelatinized starch, having high water-absorption properties, is used with sands containing up to approximately 5 per cent water. The sand may be either dried first and the requisite water added (this method gives consistent water content) or moist sand as received may be used, but with some control whereby the water ratio will be in the region of 4–5 per cent. Alternatively, there is a fully soluble dextrine, having low water absorption, for use with sands containing approximately only 1 per cent moisture. Both types of starch product enjoy a wide usage, having a total consumption in the U.K. of upwards of 12,000 tons/yr.

A recent development in the preparation of foundry core sands is the application of ' Dexecor ', special corn sugar (derived from maize starch) for imparting dry strength to cores after baking. In such application, the sugar replaces oils or resins, with a consequent reduction in either the baking time or temperatures; a saving of from 30–50 per cent in baking time is claimed by the use of this material as compared with conventional drying oils.

Moulding sands. Traditional procedure is to employ clay as the plasticizing medium in moulding sands; moreover, there are numerous deposits of sands containing quartz or clay, naturally blended in proportions which are well suited for moulding requirements. Notwithstanding this suitability, which meets the needs of many foundries, coupled to which is the low cost of naturally clay-bonded sands and indeed of added clay, there are many features in modern conditions, e.g. complexity of casting designs and high temperature of metal poured into moulds, which make it desirable to impart greater strength to sand mixtures than clay alone is capable of giving. Starches possess the property of reinforcing the strength of clay and also impart other desirable features to such sands. For these reasons the use of starches in moulding sands has already become regular practice in a great many foundries and such technique continues to expand.

Oil-Well Drilling and Instant Puddings

Oil-well drilling utilizes pre-gelatinized starch, and this has greatly improved the very deep drilling. A carefully compounded mixture of sand, water, and gelatinized starch is continuously pumped down the well. The starch swells and absorbs water. Thus it prevents loss of water from the mud on its long trip to the surface by sealing the crevices in the wall. The purpose of circulating the prepared slurry is to cool the drilling bit and push away the cuttings to the surface. Prior to the use of starch to seal the walls, great difficulties were experienced due to increasing viscosity of the circulating slurry by loss of water in porous drillings.

In the preparation of ' instant dessert ', the prime requirement is a starch requiring no further cooking and one which will convert coagulated milk protein into a soft but firm gel. By adding sodium tetrapyrophosphate to fresh cold milk, the protein is coagulated within a few minutes. If the tetrapyrophosphate is incorporated in a suitable pre-gelatinized starch, together with colour and flavour, then the starch rapidly absorbs the water in the milk and ensures a satisfactory food product. The choice of starch depends on the texture and degree of gelatinization required.

ADHESIVES

It is proposed to refer here only to the limited section of the adhesives field using starches and modified starches, making no reference to the many other types of gums and glues based on dextrines and synthetic resins, etc.

Starch and flour pastes have been used for many years for home-decorating, bill-posting, carton, box, and paper-bag making, laminating, spiral tube winding, wallpaper manufacture, and many similar applications. Even if adequately preserved the working life of starch and flour pastes is limited to a few weeks due to such factors as retrogradation and syneresis. In many applications, these storage objections are overcome by the use of dry powders which can be made down in paste form as required, but for others modified pastes have been developed for use in the prepared ' ready to use ' form. A typical example of the use of the dry powder form is in the laminating of Jacquard cards composed of fifteen or more layers of papers where starch will give the economy required without loss of strength, rigidity, or dimensional stability. For wallpaper pastes, manufacturers have produced hot water pastes, and hot water or cold water powders, the latter incorporating pre-gelatinized starch as the principal ingredient.

Where modified starches are required, the methods employed involve modification of the starch itself, either as a separate operation, or by chemical and mechanical means in the actual liquid-adhesive plant. The general result is to obtain adhesives increasingly higher in solids concentration at a fixed viscosity.

Alkali Conversions

Farina and sago have high water-absorbing capacities, developing high viscosities at low concentrations. By subjecting such pastes to high shearing action in the presence of caustic soda and subsequent neutralization, the pastes have as much as threefold concentration of solids for the same viscosity and are, in addition, stable on storage. Typical applications include wallpaper grounding, bottle labelling, biscuit-tin labelling, and the lamination of metal foil to paper.

Salt Conversions

By replacing caustic soda by certain hygroscopic salts and using the same type of shearing action, a class of adhesives is produced which gives more flexible films due to the higher moisture retention.

Because of the slower drying properties, this type finds application in bill-posting. This allows adequate time to slide and re-position the poster sections. Also because of the higher water-absorbing properties without loss of adhesion, posters will not come adrift in wet weather. A similar example in another field is shoe-socking where the sock must absorb perspiration without the bond being weakened.

Oxidized Pastes

By introducing oxidizing agents to a normal alkali conversion, the paste produced has excellent storage and stable viscosity characteristics, so that paper-bag making on high-speed machines is vastly improved. The ‘ short ’ salve-like uniform consistency ensures that the machine picks up the correct amount of adhesive without variation. Spiral-tube winding is another application of this type of paste. Paper or card is wound spirally on to a mandrel, the paste being constantly applied as the mandrel turns. Subsequent air drying of the tube tightens up the many plies and gives remarkable wall strength.

STARCH DERIVATIVES

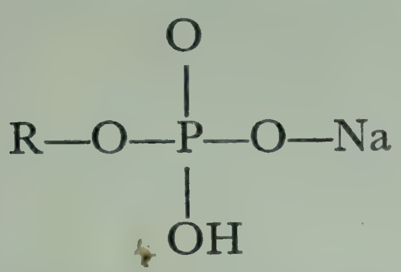
Research is constantly seeking to modify starches to produce properties which are unique, or specific in order to find new applications or improve existing ones. Reference has already been made to fractionation and genetic control yielding interesting products. Yet another avenue being actively explored is that of chemically cross-bonding starches.

Starch, with its preponderance of hydroxyl groups, is a substance which will undergo many reactions with a variety of reagents including polyphosphates, acetic anhydride, propylene oxide, and chloracetic acid. Many of these reactions and the products resulting from them are being examined for future use and some, such as the starch ethers and starch phosphates, are already available.

Starch Phosphates[7]

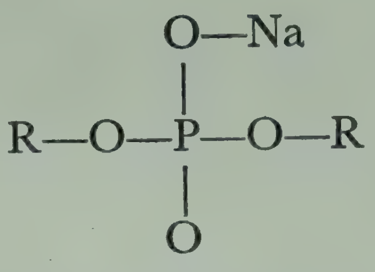
Starch will react with suitable water-soluble phosphates in two ways. With sodium triphosphate, it will produce a ‘ monostarch phosphate ’; by treating maize starch at pH 9 with water-soluble sodium metaphosphate, a cross-linked ‘ distarch phosphate ’ is produced.

If the formula for starch is represented simply as ROH, then :



$$\begin{array}{c} \mathrm{O} \\ | \\ \mathrm{R{-}O{-}P{-}O{-}Na} \\ | \\ \mathrm{OH} \end{array}$$

monostarch phosphate



$$\begin{array}{c} \mathrm{O{-}Na} \\ | \\ \mathrm{R{-}O{-}P{-}O{-}R} \\ | \\ \mathrm{O} \end{array}$$

distarch phosphate

If the monostarch phosphate is produced from maize by such esterification, then the paste properties have higher clarity and longer flow than normal maize and much more resemble farina and tapioca. Like these starches, the maize monophosphate will tend to deteriorate on prolonged cooking more readily than untreated cereal starch.

When the cross-linking reaction proceeds, the viscosity of the paste reaches a peak and then declines to very low values. Simultaneously, shortness and opacity increase due to a progressive decrease in the ability of the starch to absorb water. Finally, in the last stages of cross-linking, the starch becomes insoluble and will not gelatinize even in boiling water. Such a product is of academic interest primarily, but one application is in the dusting of surgical gloves, which may be sterilized without loss of lubricating value.

Starch Ethers

Reference has already been made to this class of derivatized starch and its uses. The general methods of manufacture covered by patents include slurrying the starch (which may itself by modified) in alkali, and treating with ethylene oxide or propylene oxide.[8] The by-products of the reactions are readily recovered by washing the slurry. An alternative cold-water-soluble starch ether is prepared by roll drying the pasted product.[9] This has the disadvantage that the salts cannot be washed out. The original patent of Penick and Ford[10] was arrived at by producing a starch with the properties of waxy starch as an alternative to genetic control.

Important amongst the starches of the future are the ionic starches, which are the outcome of the desire to improve the normal flocculating and dispersing properties of common starches. The anionic starch sulphate and cationic substituted-ammonium starches show special dispersing, flocculating, and adsorptive effects.

Polyoses

Another interesting development is the production of polyoses[11] which are soluble carbohydrate polymers obtained by chemically polymerizing glucose. They are prepared by converting starch into glucose by normal acid hydrolysis, then adjusting the pH, and concentrating the syrup, followed by adding some 2 per cent formaldehyde by weight. The mixture is dried by the usual methods and polymerization takes place on heating the dried powder after acidification in a manner similar to the manufacture of dextrines. Solutions are prepared by dispersing the light-cream to tan coloured powders, preferably in water at 70°. In solution, the polyoses are characterized by sparkling clarity, excellent stability, and a high degree of compatibility with other water-soluble products. Up to 3 per cent borax can be added to polyose solutions to improve tack.

Development of applications for polyoses is currently being directed to re-moistening gums and mucilage formulations, extenders for polyvinyl alcohol and animal glue, and as additives for viscosity stabilization of dextrines and starches.

ACKNOWLEDGEMENTS

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12

THE ENZYMIC SYNTHESIS AND DEGRADATION OF CELLULOSE AND STARCH

OF the two polysaccharides under review the enzymic synthesis and degradation of starch is much the more fully understood and for this reason will be considered first. For example, the synthesis of the chain-forming linkage of starch was reported[1] in 1940 whereas the *in vitro* synthesis of cellulose by a microbial enzyme was reported for the first time only a few months ago and the synthesis by a plant enzyme has yet to be described. Similarly the enzymes which degrade starch are much more adequately characterized than are the cellulases. Starch is perhaps the easiest and cellulose among the most difficult of polysaccharides to investigate. Starch has the advantages of reasonable solubility and being readily detected with iodine, cellulose the disadvantage of insolubility.

This review of starch synthesis and degradation is confined to the actions of plant enzymes, except for certain α-amylases which are of microbial or animal origin. The reader should consult other reviews for descriptions of the animal enzymes which synthesize glycogen,[2] the animal counterpart of starch, and for animal and microbial enzymes which degrade starch.[3,4] The review of cellulose synthesis and breakdown has not been limited in this way.

SYNTHESIS OF STARCH

The structure of starch is reviewed elsewhere in this volume.[5] It is sufficient here to mention that most starches are heterogeneous, containing two components, amylose and amylopectin. Amylose is an essentially linear molecule containing up to several thousand α-glucose units joined through 1 : 4 bonds, while amylopectin, also having a high degree of polymerization (DP), consists of short chains (average DP 20–30) of 1 : 4-linked α-D-glucose units joined in a tree-like network through α-1 : 6 bonds. Small proportions of α-1 : 3 bonds, ester phosphate groups attached at $C_{(6)}$, and possibly 1 : 2 bonds are also present, but since nothing is yet known of their synthesis and degradation, except for the hydrolysis of phosphate groups, no further mention of these will be made.

Synthesis of the α-1 : 4-*Glucosidic Linkage*

The enzyme, phosphorylase, which synthesizes the chain-forming links of starch, was discovered because of the ease with which this synthesis can be reversed. The enzyme was first found in frog and rabbit muscle.[6] Glycogen, the animal counterpart of amylopectin, was incubated with rabbit-muscle extract and phosphate buffer and the acid-labile α-D-glucose 1-phosphate (Cori ester) was formed. The same enzyme was then found in peas and potatoes.[1] (Unless stated otherwise all subsequent references to phosphorylase are to the potato enzyme.) However, no synthesis took place when the ester was incubated with the plant enzyme. It was necessary to add a preformed starch molecule as a ' primer '; synthesis then took place with the simultaneous release of inorganic phosphate. At pH 7, equilibrium was reached when the ratio of inorganic phosphate to glucose 1-phosphate was 3·1 : 1. Later investigations have shown that the smallest starch-like molecule which will ' prime ' the reaction is maltotriose, although its efficiency is much less than that of the next higher α-1 : 4-linked polyglucose homologue, maltotetraose. The higher homologues have priming activities comparable with that of maltotetraose.[7–9] It seems essential that a primer should contain α-1 : 4-linked glucose units. The only apparent exception to this generalization is that a dextran (mainly α-1 : 6-linked polyglucose) had priming activity[10] but there was no evidence that it was devoid of α-1 : 4 links.

The synthetic ' starch ' was found to be amylose,[1] which at that time (1940) had just been characterized as a distinct structural entity. The function of the primer was recognized to be as a recipient of α-glucose units transferred from the phosphate ester. The failure of the enzyme to act without the primer meant that glucose cannot be transferred from one ester molecule to another, only to a primer containing 1 : 4-linked α-glucose units, as shown in Figure 1. The point of attachment of the transferred glucose unit to the primer is the $C_{(4)}$ hydroxyl group of the non-reducing end unit of the primer. That it is not the reducing end which is the point of contact is proved, for example, by the fact that maltopentaose in which the reducing end unit has been oxidized to gluconic acid or reduced to sorbitol is still a primer.[10a]

We may describe the synthesis of amylose in terms which are applicable to almost every type of carbohydrase action (see references 3 and 11 for a fuller discussion). Each reaction of this type involves a donor substrate (D—OR) and an acceptor substrate (H—OA) (equation 1). The donor substrate is always a carbohydrate and may also function as an acceptor. The acceptor, besides being a carbohydrate, can be an alcohol, a phenol, water, etc.

$$\text{D—OR} + \text{H—OA} \rightleftharpoons \text{D—OA} + \text{H—OR} \quad \ldots (1)$$

The splitting of the donor substrate occurs usually, but not always, between a carbon atom at a reducing centre and the inter-linking oxygen atom to which the carbon is joined (glycosyl-oxy scission, cf. Figure 1). The carbohydrase is thought to participate in the reaction by first reacting with the donor (equation 2) forming an enzyme-donor complex which in turn reacts with the acceptor, regenerating the enzyme (equation 3).

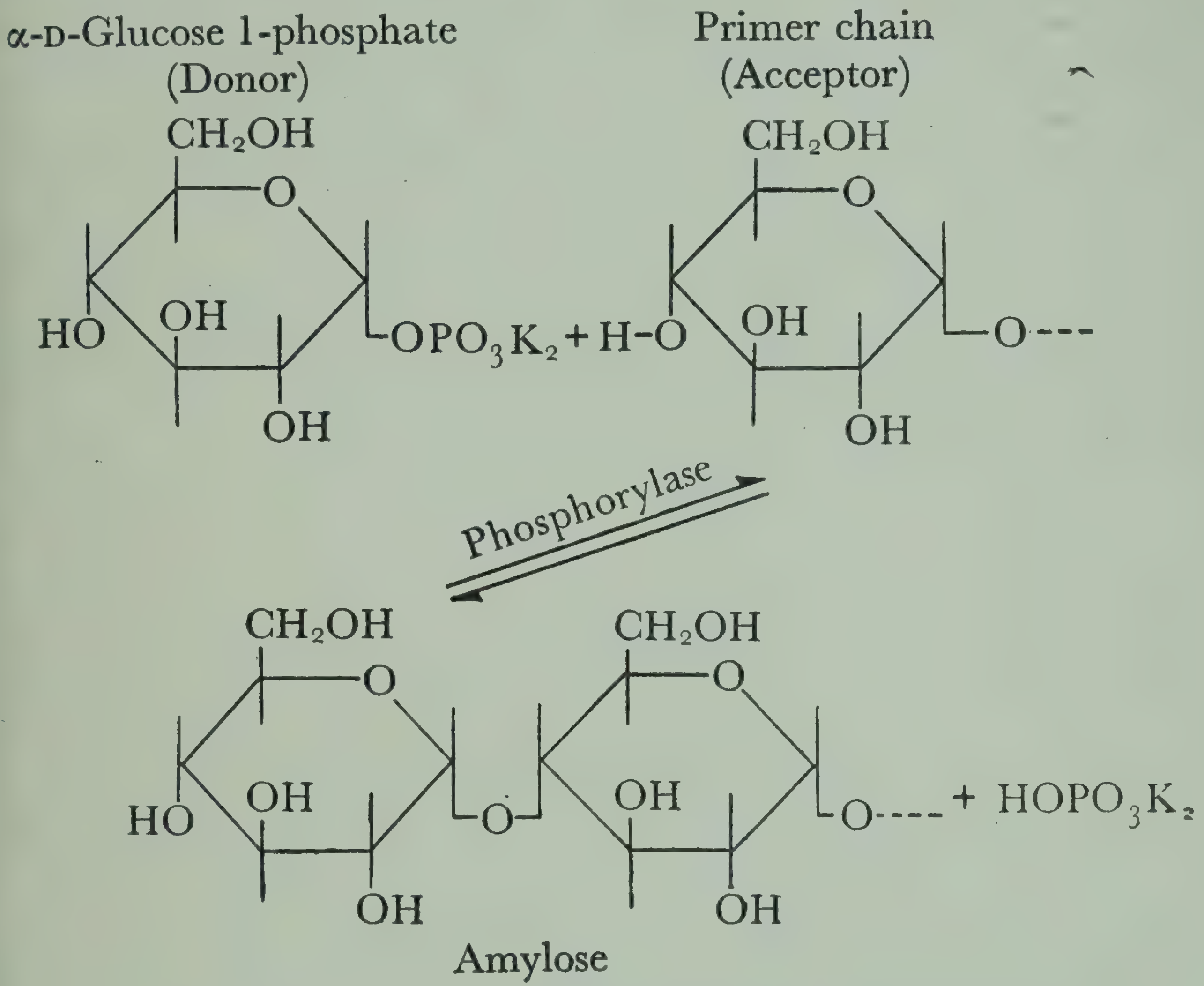

Figure 1. Unit step in the synthesis of amylose by phosphorylase

$$\text{D—OR} + \text{Enzyme—H} \rightleftharpoons \text{Enzyme—D} + \text{H—OR} \quad \ldots (2)$$

$$\text{Enzyme—D} + \text{H—OA} \rightleftharpoons \text{D—OA} + \text{Enzyme—H} \quad \ldots (3)$$

In amylose synthesis, glucose 1-phosphate is the donor and the primer is the acceptor. In phosphorolysis of amylose, the polysaccharide is the donor and phosphate ion the acceptor. Phosphorylase is highly specific. Mention has already been made of its specificity towards primer and no sugar phosphate other than α-glucose 1-phosphate functions in the reaction.[11,12] Arsenate can, however, replace phosphate ions in the degradation of amylose.[13] In this case the product of reaction is glucose. It is thought that α-glucose 1-arsenate is formed but that it undergoes spontaneous decomposition.

The manner in which phosphorylase distributes glucose units between primer molecules during amylose synthesis has been examined.[7] Two possibilities were considered. Firstly, single-chain action,[14] in which the enzyme was envisaged as causing the primer chains to grow one at a time to large molecules; secondly, multi-chain action,[14] in which the chains were considered to grow at approximately equal rates. It was proved[7,15] that multi-chain action took place at all temperatures in the range 0–55°, contrasting with β-amylase action, which partakes of a mixture of both types of action (see later). This information made possible the synthesis of amyloses of known average DP and known homogeneity,[7] which proved of value in studying the action patterns of other starch carbohydrases.

Synthesis of the α-1 : 6-*Glucosidic Linkage*

If we assume that phosphorylase creates the α-1 : 4-glucosidic linkages of both amylose and amylopectin, then to complete the synthesis of starch we need only find the enzyme responsible for the synthesis of the α-1 : 6-branch linkages of amylopectin. This is an over-simplification since it neglects the presence in starch of small proportions of other groupings (see above) and also it is not necessarily true that phosphorylase alone synthesizes both the amylose and amylopectin α-1 : 4 bonds (see later).

The synthesis of the branch linkages was first accomplished[16] in 1944, with Q-enzyme from the potato. This has the property of converting amylose into amylopectin; a mixture of phosphorylase, Q-enzyme, glucose 1-phosphate, and primer also produces amylopectin (Figure 2). The action on amylose is characterized by a rapid fall in intensity of iodine stain with very little production of reducing power (Figure 3).[17] The synthetic amylopectin was very similar to the natural material. Unlike phosphorylase action, Q-enzyme action is irreversible, but it has been postulated that Q-enzyme might be able to reposition the branch links, once they are formed.[3] It was at first thought that Q-enzyme hydrolysed amylose into short chains having the same average DP (about 20–30) as the unit

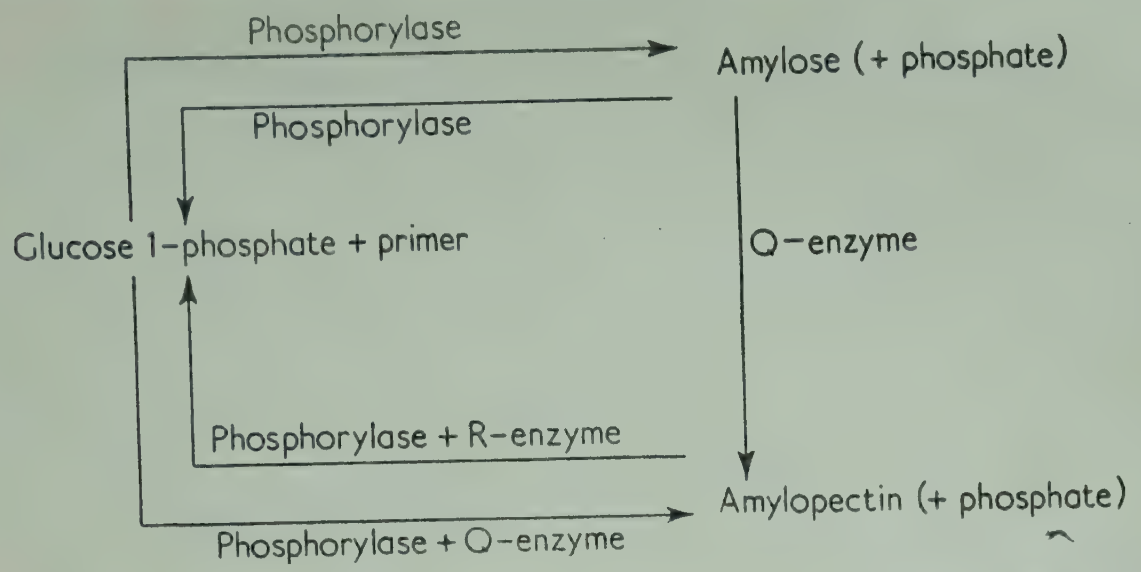
Phosphorylase
Amylose (+ phosphate)
Phosphorylase
Glucose 1-phosphate + primer
Q-enzyme
Phosphorylase + R-enzyme
Amylopectin (+ phosphate)
Phosphorylase + Q-enzyme

Figure 2. Synthesis and degradation of amylose and amylopectin as mediated by phosphorylase, Q-enzyme, and R-enzyme

chains of amylopectin, and that these were then cross-linked. Hestrin,[18] however, suggested that the conversion of an α-1 : 4 link into the α-1 : 6 link was a direct process, not involving hydrolysis. This type of linkage scission followed by simultaneous reformation has acquired the names ‘ transglycosylation ’ (glycosyl-oxy scission) and ‘ transglycosidation ’ (glycosidic-carbon scission), according to whether or not the oxygen of the split linkage is transferred (see above). It is not known which type is involved in Q-enzyme action. Hestrin's suggestion was proved correct when it was found that Q-enzyme did not cross-link 20-unit chains.[19] By allowing Q-enzyme

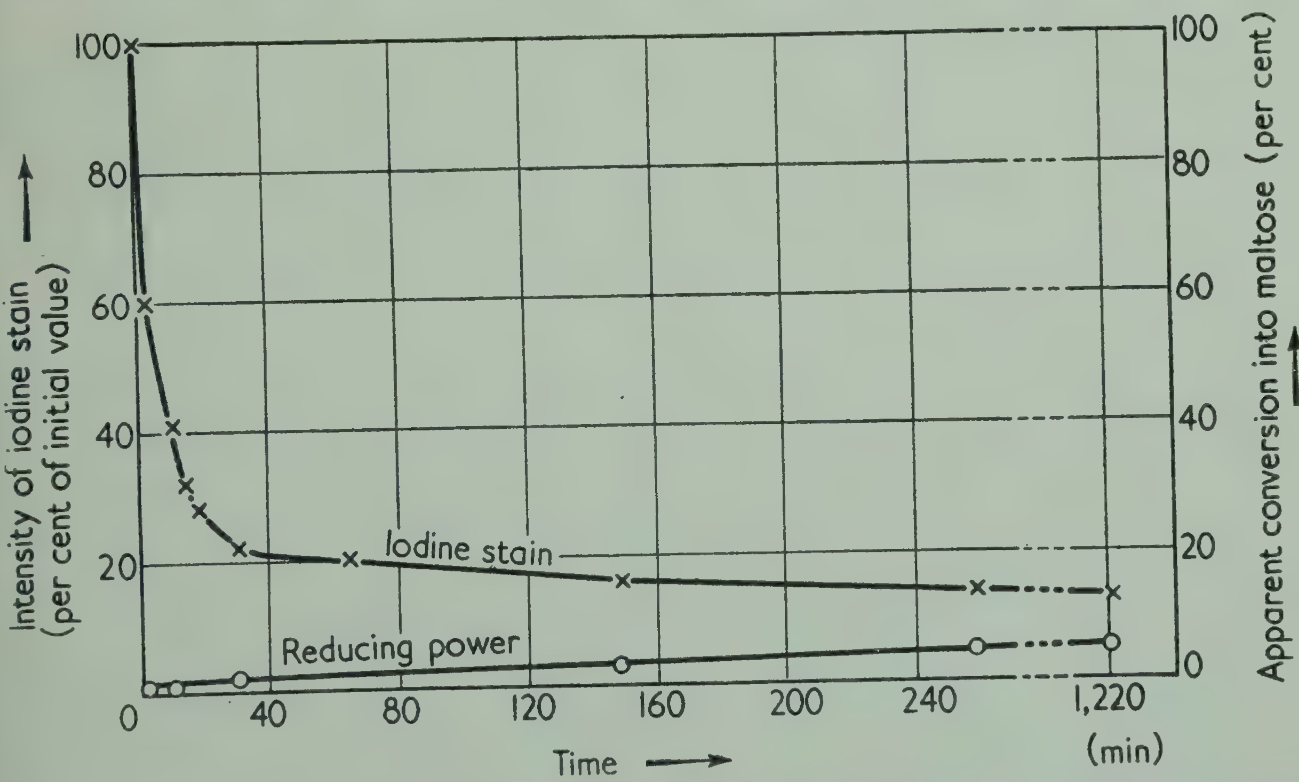

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Figure 3. Action of Q-enzyme on amylose. Changes in intensity of iodine stain and in reducing power

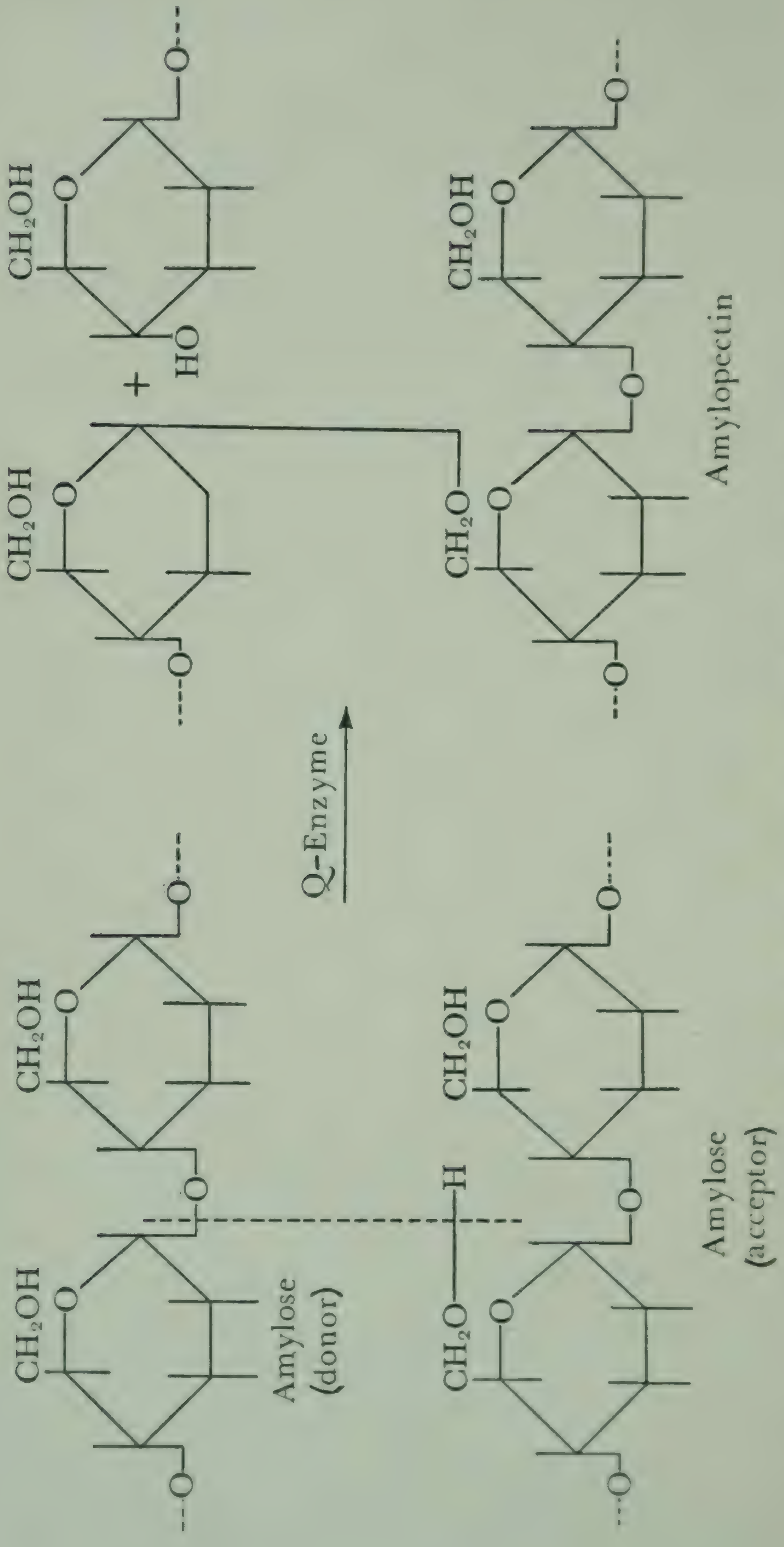
CH2OH
O
HO
+
Q-Enzyme
Amylose (donor)
Amylose (acceptor)
Amylopectin
H
CH2O

Figure 4. Unit step in the conversion of amylose into amylopectin by Q-enzyme

to act on a range of synthetic amyloses the minimum DP of chain on which rapid branching action was found to take place was 40.[20] The mechanism of Q-enzyme action is shown in Figure 4. It is supposed that the enzyme reacts with an amylose chain (donor, DP $\nless$ 40), detaching a segment, with which it forms a complex. This complex then reacts with a primary hydroxyl group in another amylose chain (acceptor), when the α-1 : 6 link is created and the enzyme set free again. Exactly what requirements potato Q-enzyme has towards acceptor molecules are not known. Perhaps even maltose could function in this way, as it can for the similar enzyme from the protazoon *Polytomella coeca*.[21]

The role of D-enzyme in Starch Metabolism

Phosphorylase creates α-1 : 4 glucosidic bonds from the phosphate bonds of the Cori ester. Q-enzyme creates α-1 : 6 bonds from α-1 : 4 glucosidic bonds. A third enzyme in the potato, D-enzyme, has no power to create or destroy linkages, but is a transglycosylase (or transglycosidase), acting on α-1 : 4 bonds by breaking them and reforming them in new positions. It does not act on α-1 : 6 linkages.[22] For example, D-enzyme converts maltotriose into an equimolecular mixture of maltopentaose and glucose by a reversible process in which a maltose residue is detached from one maltotriose molecule (donor) and added to a second molecule (acceptor) (Figure 5). The maltose residue transferred comprises the non-reducing end and the central glucose unit of maltotriose, and these are attached to the non-reducing end unit of the acceptor.[23] This transferring action takes place at random, except for two restrictions. The enzyme cannot act on an α-1 : 4 link at the non-reducing chain end of a maltodextrin, nor on the link penultimate to the reducing end.[23a] Maltose is therefore not a donor molecule but can act as an acceptor, as also can other maltodextrins, starch, glucose, and monosaccharides of similar structure to glucose, such as D-xylose, D-mannose, and L-sorbose.[24] It has yet to be proved that D-enzyme functions in the *in vivo* synthesis of starch, but on the assumption that this is so, its presence along with phosphorylase and Q-enzyme may help to explain some outstanding problems. Firstly, there is the problem

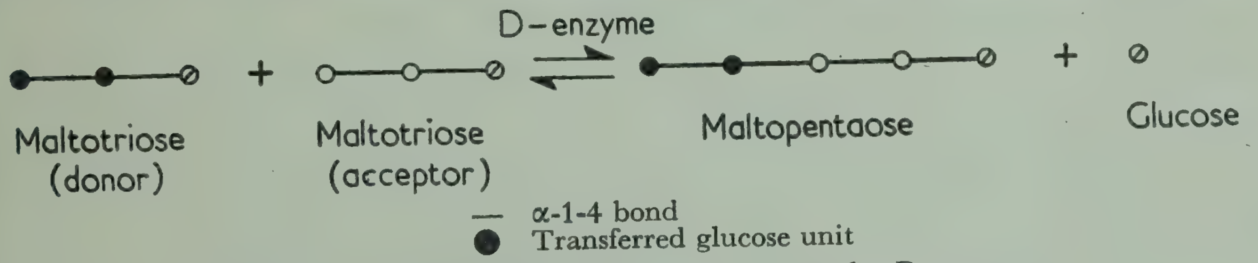

Figure 5. Disproportionation of maltotriose by D-enzyme

of the origin of the primer from which starch is synthesized. Phosphorylase cannot create its own primer but in the presence of D-enzyme and a supply of glucose, amylose in process of synthesis by phosphorylase can be transferred in segments to the glucose, creating new primer molecules. In the reverse process of phosphorolysis, phosphorylase does not reduce the length of an amylose chain to fewer than 4 glucose units (maltotetraose). D-enzyme converts maltotetraose into larger maltodextrins which are capable of being phosphorolysed. The conversion of starch into glucose 1-phosphate is therefore appreciably more complete when D-enzyme is present. For example, phosphorylase has no phosphorolytic action on maltotriose. When D-enzyme is added, a conversion into 2 molecules of glucose 1-phosphate and 1 molecule of glucose takes place.[24a]

Another very important problem is that of explaining how it is that amylose and amylopectin are found together in most starch-bearing plants. The existence of amylose seems to represent an incompleteness of action of Q-enzyme. Why should such action be incomplete and why is the ratio of amylose to amylopectin so very constant for a given plant at maturity? There is some evidence for a change in the ratio during the ripening of barley[25] and maize,[26] but in the potato it was found to be constant at several stages of growth.[27] The answer may be that amylose and amylopectin are synthesized in compartments separated by a membrane which permits the interdiffusion of oligosaccharides and sugar phosphates, but not of enzymes and polysaccharides. Such a system has been described,[3] in which it is suggested that a maltodextrin (maltotetraose) could be converted into a 3 : 1 mixture of amylopectin and amylose by the agencies of D-enzyme, Q-enzyme, phosphorylase, phosphoglucomutase and hexokinase. The ratio is that commonly found naturally. Although this scheme is a practical means for the *in vitro* synthesis of amylose and amylopectin there is no evidence that it in fact functions *in vivo*. While one attempts to explain starch synthesis in terms of known enzyme systems there can be no assurance that alternative systems will not be discovered. Phosphorylase was long thought to be responsible for amylose synthesis but the more recently discovered D-enzyme now provides an alternative. Any consideration of starch synthesis should also bear in mind the known and apparently intimate connection of the metabolism of this polysaccharide and that of sucrose.[28] An enzyme, amylosucrase, is known which can synthesize a starch-type polymer from sucrose,[29] and although it has not yet been obtained from plants the possibility of its presence there is suggested from time to time and there is some circumstantial evidence for believing it to be present in leaves.[30] Yet another possibility has been raised by a recent report that glycogen can be synthesized from uridine diphosphate glucose (UDPG) (Figure 6). The enzyme, from liver of unspecified origin, caused the formation of glycogen and uridine diphosphate from UDPG, with glycogen or starch as primer,[31] and seems to be distinct from the already known animal phosphorylase, which failed to synthesize glycogen from

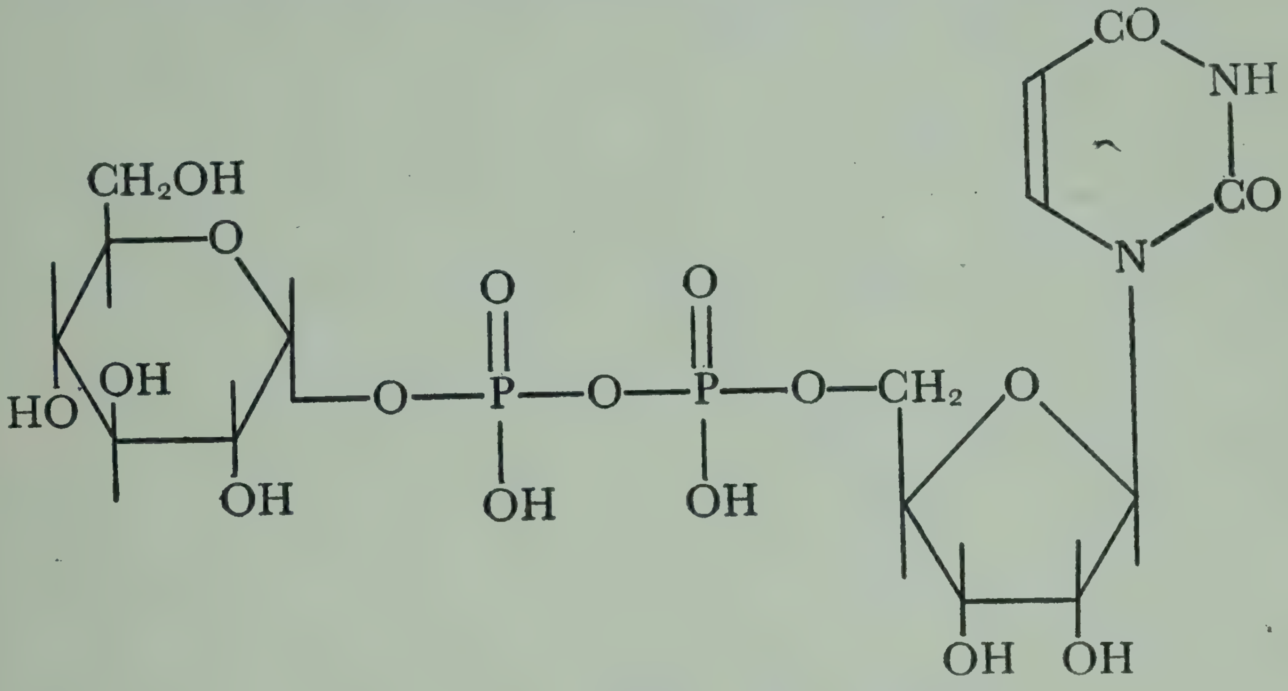
CH₂OH
O
HO
OH
OH
O
P
OH
O
P
OH
O
CH₂
O
CO
NH
CO
N
OH
OH

Figure 6. Uridine diphosphate glucose (UDPG)

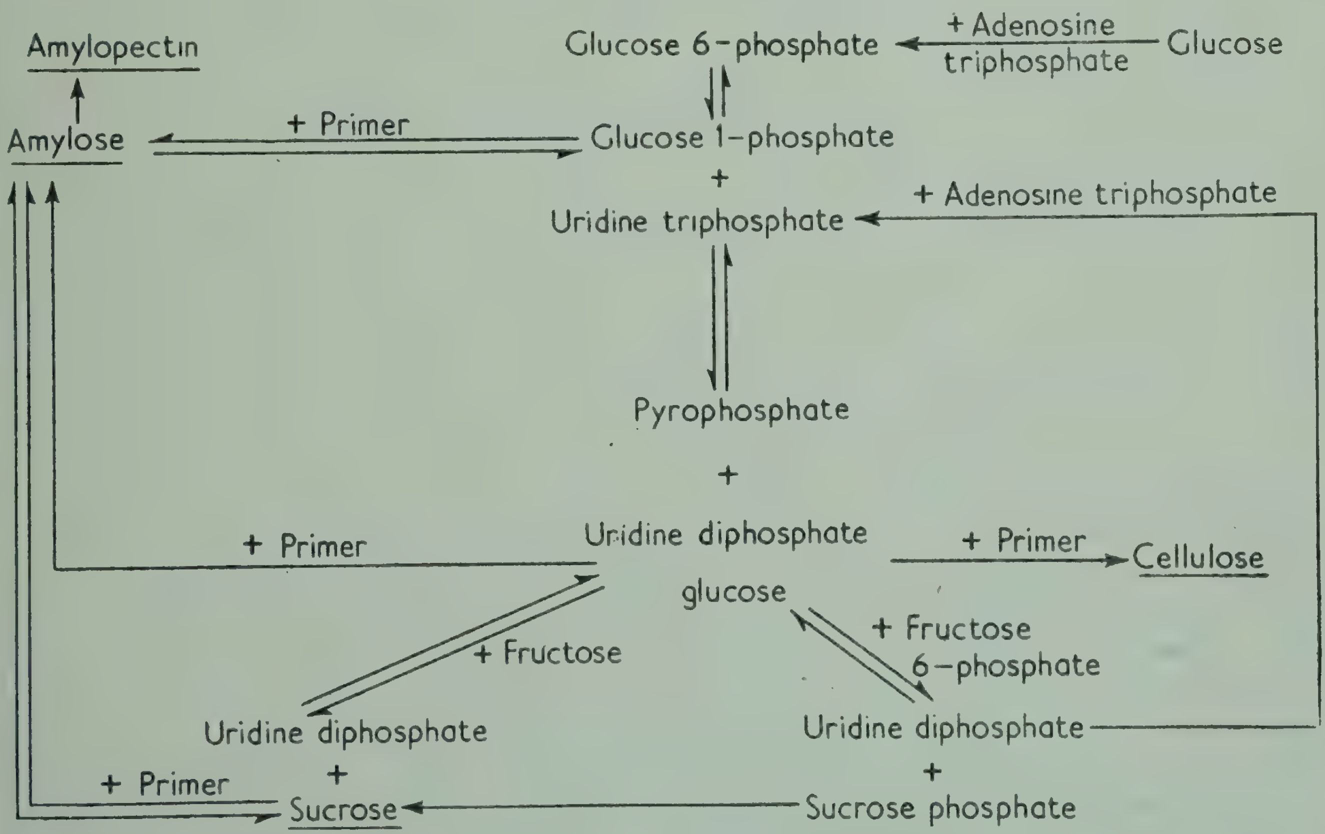
Amylopectin
Amylose
+ Primer
Glucose 6-phosphate
+ Adenosine triphosphate
Glucose
Glucose 1-phosphate
+
Uridine triphosphate
+ Adenosine triphosphate
Pyrophosphate
+
Uridine diphosphate glucose
+ Primer
+ Primer
Cellulose
+ Fructose
+ Fructose 6-phosphate
Uridine diphosphate
+
Sucrose
+ Primer
Uridine diphosphate
+
Sucrose phosphate

Figure 7. A summary of some of the known pathways of synthesis of sucrose, starch, and cellulose. (Note. Glycogen, not amylose, is the product isolated from synthesis with UDPG[31] and sucrose.[29] It may be assumed, however, that amylose is first synthesized and that a second enzyme introduces the branch linkages[29] (cf. Q-enzyme))

UDPG.[32] Korkes[33] postulated that UDPG was involved in rabbit phosphorylase action and that glucose 1-phosphate was converted into glycogen via this nucleotide. Uridylic acid had been isolated from the crystalline enzyme,[34] although in the recent report of the isolation of pyridoxal-5-phosphate from crystalline muscle phosphorylase the statement is made that no other nucleotides were detected.[35] UDPG is already known to be involved in the synthesis of sucrose in wheat seedlings[36] and is itself synthesized from uridine triphosphate and glucose 1-phosphate.[37] There are, therefore, three potential sources of the glucose units of starch, namely, glucose 1-phosphate, UDPG, and sucrose (Figure 7). The argument for regarding glucose 1-phosphate as the substrate for starch synthesis *in vivo* therefore becomes less secure. It may only be an intermediate in the phosphorolytic breakdown of starch.

DEGRADATION OF STARCH

Scission of α-1 : 4 *Linkages*

There are four types of enzyme capable of transforming the α-1 : 4 bonds of starch. Three have already been mentioned. Phosphorylase can convert starch into α-glucose 1-phosphate, the 1 : 4 linkage being transformed into an ester-phosphate bond. Q-enzyme converts α-1 : 4 links into α-1 : 6 links. D-enzyme, or related transglycosidases such as amylomaltase or *Bacillus macerans* amylase,[3,11,37a] repositions the linkages. The fourth type is represented by the amylases which use water as the acceptor molecule and therefore differ from the other three types of enzyme in liberating reducing sugars. This class of enzyme is capable of subdivision into exo and endo enzymes. The exo enzymes split starch by an endwise action; the endo enzymes, like hydrogen ions, split starch by random fragmentation.

Endo enzymes. An alternative name for this class of enzyme is α-amylase, the prefix denoting that the reducing group is set free in the α-configuration.[3] This enzyme pervades most living tissue, often in only small amounts but frequently in large quantity, as in germinating seeds, the pancreas of animals, and human saliva, wherever, in fact, starch is required to be digested. Many α-amylases have been crystallized.[3] As far as is known, the specificity of the enzyme towards α-1 : 4 linkages is absolute.

α-Amylase action on starch is characterized by a rapid fall in viscosity, a rapid disappearance of iodine stain, and the eventual development of a large reducing power, owing to the fragmentation of starch into small oligosaccharides. The various α-amylases differ from each other in two main respects. Firstly, animal α-amylases require chloride ions as activator, whereas plant α-amylases require calcium ions. Secondly, the enzymes show differences in their relative affinities for starch and small oligosaccharides. For example, while human salivary α-amylase readily converts amylose into maltose and maltotriose,[38] and eventually, by slow hydrolysis of the latter, into maltose and glucose,[39] barley-malt α-amylase action becomes relatively slow when the oligosaccharide products have DP about 6. These substances are hydrolysed only slowly, the ultimate products being maltose and glucose.[3,4,40] The differences may be attributed to the varying affinities of the enzymes for linkages in the vicinity of the chain ends. Linkages remote from the ends are probably attacked at random and with equal ease by both types of α-amylase. Human α-amylase displays a low affinity only to the terminal non-reducing and reducing-end linkages,[38,39] but malt α-amylase is very inefficient in hydrolysing several linkages in sequence from the non-reducing chain end, accounting for its low affinity for the small maltodextrins.[3,4,40,41] On the other hand, it has more affinity for the reducing-end linkage than has salivary α-amylase and splits glucose directly from a range of maltodextrins, whereas the glucose produced by salivary amylase arises almost wholly from maltotriose, which is devoid of the internal linkages that this amylase prefers to attack. Other α-1 : 4 linkages for which the α-amylases have little or no affinity are those in the vicinity of the α-1 : 6-branch points of amylopectin. Some of these 1 : 4 linkages are completely immune to α-amylase and in consequence the α-1 : 6 bonds of amylopectin are not released as part of the α-1 : 6-linked diglucose, isomaltose, but as part of larger amylase-resistant oligosaccharides (α-limit dextrins), still containing α-1 : 4 bonds. The smallest such oligosaccharide formed by malt α-amylase is the trisaccharide panose[42] (see p. 69). The smallest α-limit dextrins produced by salivary α-amylase are a tetrasaccharide and a pentasaccharide. The tetrasaccharide consists of α-glucose joined through a 1 : 6 link to the non-reducing end of a maltotriose molecule.[43] In the pentasaccharide, α-maltose replaces the α-glucose.[43a]

Exo enzymes. The chief example of this type is β-amylase, found only in plants, notably in ungerminated cereals, soya beans, and sweet potatoes.[3,4,40] The sweet-potato enzyme was the first plant carbohydrase to be crystallized.[44] The enzyme acts on starch chains by an end-wise approach from the non-reducing chain end, successively splitting off maltose units by hydrolysis of alternate linkages. The maltose is liberated in the β-form,[3] i.e. hydrolysis of the α-linkage is accompanied by Walden inversion, in contrast to α-amylolysis. The action of the enzyme is arrested when it approaches any glucose unit substituted other than at $C_{(1)}$ and $C_{(4)}$: for example, at branch points, or at glucose units esterified with phosphate at $C_{(6)}$ such as occur in amylopectin.[3,4,40] The branch linkages are not exposed by the enzyme as terminal bonds; action ceases when the enzyme approaches within 1–3 glucose units from the branch point.[45,46] Amylopectin is therefore only incompletely degraded by β-amylase. The yield of maltose varies from 50–70 per cent, depending on the plant source of the amylopectin. The α-1 : 4 linkages lying with respect to the branch points on the side remote from the non-reducing chain ends are immune from attack by β-amylase, but not, of course, by α-amylase. The difference in degree of β-amylolysis of amylose and amylopectin has been one of the best and easiest means of distinguishing between the two polysaccharides, for amorphous preparations of the enzyme cause a complete conversion of amylose into maltose. From this it was concluded that amylose is a linear molecule containing no glucose units substituted other than at $C_{(1)}$ and $C_{(4)}$. The discovery that crystalline β-amylase, although showing no difference from the amorphous enzyme as far as amylopectin was concerned, yielded only about 70 per cent of maltose from amylose caused a revision in ideas on the structure of this polysaccharide. It was found that 2 enzymes were needed for the complete hydrolysis of amylose, namely β-amylase and Z-enzyme.[47] The latter enzyme removes the anomalous groupings which impede the action of β-amylase. It can be obtained free from β-amylase and exerts its action independently of the amylase. Evidence has been presented that Z-enzyme is a β-glucosidase[47] but this has been contested.[48]

As with phosphorylase, the problem has been considered whether β-amylase acts by a single-chain or multi-chain mechanism. Much work has been done on this problem and only recently has some agreement been reached.[15,49,50] It seems that the enzyme can remove more than 1 maltose unit during each encounter with a substrate molecule and it is likely that the number of units removed increases with the size of the substrate molecule. That is, small maltodextrins are attacked largely by a multi-chain mechanism (1 maltose unit removed per encounter) but natural potato amylose, having a DP of up to 6,000, is attacked by a single-chain mechanism. A partial explanation of this phenomenon is that a large molecule is more sluggish in its movements than a small molecule, and in consequence is more likely to suffer repeated attacks, once it has been encountered by the enzyme. The catalytic activity of β-amylase is high. Under optimum conditions, 1 molecule of enzyme hydrolyses 237,000 bonds per minute.[51,52] Temperature and pH dependences of the action pattern have been noticed with maltodextrins and synthetic amyloses of DP up to 50. The proportion of single-chain action is greatest under optimum conditions for enzyme activity and least under unfavourable conditions.[15]

Scission of α-1 : 6-Branch Linkages

Potatoes and broad beans contain an enzyme (R-enzyme) which hydrolyses the α-1 : 6-branch linkages of amylopectin.[53] The action of the enzyme is recognized by an increased susceptibility of the amylopectin to β-amylolysis (see above) and an increased intensity of iodine stain. It seems that the free unit chains of amylopectin can more readily form a complex with iodine than when joined together in the tree-like high polymeric molecule. The enzyme does not attack glycogen,[54] which is similar in structure to amylopectin, but the unit chains are only about half the length.[2] Steric factors are evidently involved here because splitting of the glycogen by α-amylase or by acid renders the 1 : 6 links susceptible to R-enzyme.[54] Evidently the glycogen molecule is too compact to allow penetration by the enzyme. R-enzyme has certain other specific requirements towards its substrates. It will not hydrolyse isomaltose or dextran, both containing α-1 : 6-linked glucose units. The substrates for R-enzyme must also contain α-1 : 4 bonds. It will not hydrolyse a single α-glucose unit joined through a 1 : 6 link to a maltodextrin chain. The shortest such 1 : 6-linked grouping which it will remove is maltose.[55] This is in contrast to the animal counterpart of R-enzyme, namely amylo-1 : 6-glucosidase, which removes only glucose ‘ stubs ’ and not longer chains. The smallest molecule which is attacked by R-enzyme is the pentasaccharide α-limit dextrin produced by salivary α-amylolysis of amylopectin (see above).[43,55] R-enzyme has recently been separated into two components, one of which attacks amylopectin but not the α-limit dextrins, the other having the reverse specificity.[56]

Degradation of Granular Starch

None of the isolated starch-degrading enzymes referred to in earlier sections, i.e. phosphorylase, α- and β-amylases, can degrade granular starches at a rate remotely approaching that at which dissolved starch is degraded. Yet it is known that starch disappears rapidly during seed germination and by microbial attack during digestion in animals.[3] The problem of reproducing such rapid attack *in vitro* is similar to that discussed later for insoluble cellulose, but has not received so much attention as cellulolysis. Starch is so readily soluble that the problem can be avoided, but in using dissolved starch in *in vitro* studies it must always be remembered that the conditions of such experiments are in general not those obtaining when starch is degraded *in vivo*.

In searching for the causative agent of whole starch breakdown, suspicion has naturally fallen on α-amylase. Balls and Schwimmer[57] found that fungal and pancreatic α-amylases would slowly digest starch but when mixed together the rate of digestion increased more than was expected. This synergistic action was thought to be due to the removal of maltose by a maltase in the fungal amylase preparation, maltose being an inhibitor of the α-amylolysis of starch. Gates and Sandstedt[58] were able to achieve 80 per cent digestion of granular maize starch in 20 hr by a bacterial amylase preparation and found that a cationic detergent, and sodium and calcium chlorides, stimulated amylolysis. However, when several α-amylases were tested there was no correlation between the relative rates of attack on dissolved and granular starch; the relative rates varied by a factor of 20. Earlier workers[59] had postulated that a special type of amylase (' raw starch amylase ') was involved in the solubilization of starch by malt preparations, this being different from the α-amylase which attacks dissolved starch. This view was later contested.[60] Some idea of the low rate of attack on whole starch is given by the fact that the relative rates of attack of bacterial amylase on granular and dissolved starch are[58] in the ratio 1 : 20,000.

Recent work with potato extracts has revealed that phosphorylase may be the agent of degradation of whole potato starch.[61] Inorganic phosphate accelerates the degradation and one of the main products is glucose 1-phosphate. The extracts contain a dialysable inhibitor, which may be related to chlorogenic acid, a phosphorylase inhibitor already known to be in the potato.[62]

Granules which have suffered physical damage are more easily attacked by bacteria and by cell-free enzymes, and the attack occurs at the point of damage.[63] Prolonged ball-milling of starch grains leads eventually to their becoming soluble in cold water and becoming readily attackable by amylases.[64]

SYNTHESIS OF CELLULOSE

General Considerations of the Mechanism of Polysaccharide Synthesis

Before mentioning the known facts about cellulose synthesis it is relevant to consider in general terms the conditions under which polysaccharide synthesis takes place. As will be seen later, the mechanism of cellulose synthesis in the plant is not yet understood. These remarks may therefore serve to define the probable and less probable ways in which this synthesis could take place. Speculation on this subject, if it is to have any relation to actuality, cannot stray very far from already known pathways of polysaccharide synthesis and these are indeed few in number. Apart from four examples discovered during the past year, no new type has been discovered more recently than 1950 and starch and glycogen remain the only polysaccharides of plant and animal origin whose synthesis by cell-free enzyme extracts has been demonstrated *in vitro*. The basic enzymes involved in starch and glycogen synthesis have been known since 1944 and have only been added to in number by the discovery of D-enzyme in 1953 and the UDPG-utilizing enzyme in 1957 (see above). All other known examples of polysaccharide synthesis relate to the action of microbial enzymes and only a half dozen or so polysaccharides are involved.[65]

Equation (1), which defines the action of carbohydrases, is subject to certain limitations when it applies to polysaccharide synthesis. These are limitations imposed on the nature of the donor and acceptor substrates. In the case of the donor, the limitation is that it should not also be an acceptor, e.g. glucose 1-phosphate is a donor in amylose synthesis but not an acceptor. When the donor has this dual role, the degree of polymerization of the products of transferase action cannot greatly be increased over that of the parent substrate. The action of D-enzyme on maltotriose is an example of this type (Figure 5). As we have seen, however, such a reaction can be caused to synthesize polysaccharide if the position of equilibrium is disturbed by removal of one of the products of the transferase action. This limitation on the donor need not, however, apply if it happens that a substance can be found of which the acceptor (or primer) activity is much greater than that of the donor. This new substance may in fact be the product of addition of a transferred sugar unit from one donor molecule to another. In this way a few donor molecules may be the nuclei of primers which are so efficient that they successfully absorb all the transferred sugar units, in competition with donor molecules.[37a] Two examples where such a mechanism may operate are the syntheses of dextran (1 : 6-linked poly-α-D-glucopyranose) by dextransucrase, and levan (2 : 6-linked poly-β-D-fructofuranose) by levansucrase. Both polysaccharides are synthesized from sucrose by cell-free extracts of micro-organisms and it is not necessary to add primer.[11,66,67] It has been supposed that levan synthesis may begin with the reaction shown in equation (4) (F—G = sucrose)

$$\text{F—G} + \text{F—G} \rightleftharpoons \text{F—F—G} + \text{G} \qquad \ldots (4)$$

and that the trisaccharide F—F—G is able to accept further transferred fructose residues far more efficiently than sucrose.[67] Polysaccharide synthesis proper then ensues. It has not been proved, however, that primer of a different and unknown type is not introduced as a contaminant of the enzyme, although it is of obvious significance that levan contains a small amount of glucose,[37a,68] and dextran, a small amount of fructose,[66] and that levan- and dextran-derived[66,67,69] oligosaccharides can accept sugar units transferred from sucrose.

The limitation imposed on the acceptor is that it cannot be water, that is, the enzyme must not be a hydrolase. An enzyme such as α-amylase, catalysing reactions in which water is apparently the only acceptor, cannot of course be considered as an agent of polysaccharide synthesis, but some enzymes can use both sugars and water as acceptors. Yeast invertase (β-fructofuranosylase) is such an enzyme. It hydrolyses sucrose to glucose and fructose but also forms tri-, tetra-, and even higher oligo-saccharides by the process shown in equation (4).[37a] This type of action is reversible, whereas the transfer to water, causing the dissipation of the energy of the sucrose linkage in the form of heat, is essentially irreversible.[70] As a consequence all the synthetic oligosaccharides are ultimately hydrolysed to glucose and fructose. Incidentally, the fact that an enzyme causes an increase in reducing power during its action does not necessarily mean that it is a hydrolase. Both dextransucrase and levansucrase liberate 1 free reducing monosaccharide molecule from sucrose, in the form of glucose and fructose respectively, for each glycosidic linkage synthesized.

Other limitations on the nature of the donor and acceptor could also be defined but, provided that the two mentioned are satisfied, polysaccharide synthesis between donor and acceptor will result. The position of equilibrium in such a synthesis will depend on the difference in free energy between the bond split in the donor and that synthesized between the transferred sugar unit and the acceptor. If there is little or no change in free energy then polysaccharide, as opposed to oligosaccharide, synthesis will not take place. Such a situation is exemplified by D-enzyme action. In the case of phosphorylase action, however, the energy difference between the glycosidic linkage of α-glucose 1-phosphate and of amylose is such that the position of equilibrium is very much on the side of amylose.[1,71]

The polysaccharide syntheses mentioned may be divided into two types. In the first type, the synthetic polysaccharide grows by the attachment of 1 monosaccharide unit at a time to the primer molecule, e.g. synthesis of amylose, levan, dextran. In the second type, which may be called ' block polymerization ',[65] the enzyme transfers several monosaccharide units at a time, in the form of a chain segment, from the donor to the acceptor. Q-enzyme and D-enzyme catalyse this type of action. It is conceivable that enzymes can act on di- or tri-saccharide phosphates in analogous fashion to the action on glucose 1-phosphate or UDPG, and build up polysaccharides by increasing the DP by 2 or more units at a time. This idea is useful in exploring the mechanism of synthesis of polysaccharides containing more than one monosaccharide or more than one glycosidic linkage, particularly those polysaccharides in which there are regular repeating structural units. A case in point is nigeran, a poly-α-glucose containing alternate 1 : 3 and 1 : 4 bonds.[72] It has been suggested that this is synthesized by polymerization of a maltose derivative through 1 : 3 bonds or of a nigerose derivative (α-1 : 3-linked diglucose) through 1 : 4 bonds.[73] Other examples of this type of polysaccharide are (i) the polyglucose lichenin from *Cetraria islandica*[74,75] or from oats,[75] which may consist of regular repeating units of β-cellotriose joined through 1 : 3 bonds,[75] and (ii) hyaluronic acid,[76] which consists of alternate units of D-glucuronic acid and D-glucosamine. It should also be borne in mind that a polysaccharide containing only one monosaccharide and only one linkage, such as cellulose, might be synthesized by block polymerization and that in considering possible substrates for polysaccharide synthesis, attention should not be confined only to derivatives of monosaccharides.

Synthesis of the β-1 : 4-Glucosidic Linkage

The structure of cellulose is reviewed in Chapter 2. It is sufficient for the purposes of the discussion of cellulose biosynthesis to consider the polysaccharide as being a high-molecular-weight polymer of 1 : 4-linked β-glucose units.

A few enzyme-catalysed reactions are known which result in the formation of cellobiose and higher cellodextrins. These can be examined from the point of view of whether the conditions of synthesis could be so modified that cellulose synthesis would result. One such reaction is the synthesis of cellobiose from glucose by the action of almond emulsin (β-glucosylase).[77] This is a very slow process, being apparently dependent for synthesis on the thermal energy of the glucose molecules, and not, as is usual in oligosaccharide synthesis, gaining any energy by the previous splitting of a glycosidic linkage. It has been suggested[78] that this is a first step towards cellulose synthesis, but the extremely low rate of the reaction coupled with the fact that cellobiose is only one of 5 glucose disaccharides formed in the process, and not the major product at that, suggest that this type of reaction is not of any great importance *in vivo*. Enzymes are known which act on cellobiose and, by a process of transglycosidation, form other sugars.[78a] Of particular interest is the enzyme from *Chaetomium globosum* which forms cellotriose and glucose from cellobiose.[79]

$$\text{Cellobiose} + \text{cellobiose} \rightleftharpoons \text{cellotriose} + \text{glucose} \quad . \; . \; . \; (5)$$

This reaction is analogous to that catalysed by potato D-enzyme (see above) and just as D-enzyme is potentially capable of forming amylose, so is the *C. globosum* enzyme potentially capable of forming cellulose. Cellulose synthesis should take place if the glucose could be continually removed. By adding glucose oxidase to the reaction mixture the transferase enzyme was caused to build up higher cellodextrin oligosaccharides.[79] The enzymic synthesis of cellulose by this means is therefore feasible and further developments are awaited with interest. Nevertheless, the *C. globosum* enzyme does not create additional β-1 : 4-glucosidic linkages; it merely repositions the pre-existing linkages of cellobiose. The β-1 : 4-glucosidic linkage is, however, created in a synthesis of cellobiose from α-glucose 1-phosphate by an enzyme from *Clostridium thermocellum*[80] (equation 6). The polymerization does not seem to progress beyond cellobiose

$$\alpha\text{-Glucose 1-phosphate} + \text{glucose} \rightleftharpoons \text{cellobiose} + \text{phosphate} \quad . \; . \; . \; (6)$$

but it is a potential starting point for cellulose synthesis. The reaction is of interest in involving a Walden inversion, which is unusual. An analogous case is the reversible formation of maltose (α-1 : 4 link) from β-glucose 1-phosphate and glucose by an enzyme from *Neisseria meningiditis*.[80a]

An intensively investigated cellulose-synthesizing system is present in organisms of the *Acetobacter* species. These organisms, particularly *A. xylinium* and *A. acetigenum*, rapidly synthesize a film of extracellular cellulose on the surface of the medium and non-viable cellulose-synthesizing suspensions are easily prepared.[81,82] Soluble cellodextrins and phosphorus- and fructose-containing oligosaccharides are also formed.[83] Until recently, however, all attempts to demonstrate synthesis by cell-free preparations have failed. This has not necessarily been because the preparations were inactive. As long as the substrate for cellulose synthesis has remained in doubt the difficulty has been to know whether the enzyme preparation or the test substrate is at fault. In the last few months three preliminary reports of cellulose synthesis by insoluble cell-free fractions of *A. xylinium* have appeared. Seemingly the most important of these is the synthesis of cellulose from UDPG (donor substrate) and cellodextrins (primer or acceptor substrate).[84] The enzyme was a cell-free particulate system, sedimenting at 140,000 *g*. Radioactive cellulose was formed from radioactive UDPG in about 1·5 per cent yield. Glucose 1-phosphate was not a donor substrate. The reaction is, presumably,

$$n\text{UDPG} + \text{Primer} \rightarrow \underbrace{(\text{Glucose})_n\ \beta\text{-4 Primer}}_{\text{Cellulose}} + n\text{UDP} \quad . \; . \; . \; (7)$$

This reaction also involves a Walden inversion of the α-glucosidic linkage in UDPG.

The other two reports of cellulose synthesis are firstly the formation of cellulose microfibrils from glucose and adenosine triphosphate by an *A. xylinium* homogenate, observed under the electron microscope,[85] and secondly, the synthesis, in 15–18 per cent yield, of radioactive cellulose by a suspension of extracted *A. xylinium*, again using glucose and adenosine triphosphate.[86] The optimum pH was 8·5 and with [1-^{14}C]glucose as substrate, virtually all (96·7 per cent) of the radioactivity of glucose recovered from the synthetic cellulose was still associated with $C_{(1)}$ showing that the glucose had been polymerized without a preliminary breakdown and resynthesis.

The findings that both glucose and UDPG are substrates for cellulose synthesis are not necessarily in conflict. That adenosine triphosphate is essential for synthesis from glucose makes it highly probable that the glucose was converted into cellulose via UDPG, with glucose 6- and 1-phosphates as intermediates. Enzymes are known which effect this conversion; not all of them have yet been demonstrated in *A. xylinium.* Figure 7 illustrates these enzymic pathways and also shows the central role of UDPG in the synthesis of sucrose, amylose, amylopectin, and cellulose. This multi-enzyme system is, admittedly, a composite scheme based on observations on animals, plants, and micro-organisms. No one organism has yet been shown to contain the whole system nor is it necessarily true that such an organism will ever be found. One might think that sucrose and cellulose synthesis, for example, are not likely to take place in vertebrate animals but cellulose has recently been found in bovine collagenous tissue[87] and other unexpected findings may yet be in store.

Our knowledge of cellulose synthesis in plants is much less advanced than in *A. xylinium*. It is to be expected that attempts will now be made to demonstrate cellulose synthesis from UDPG by plant enzymes but it cannot be assumed that this is necessarily the plant substrate for this synthesis. Sucrose synthesis provides an example where knowledge of the bacterial mechanism did not solve the problem of its biosynthesis in plants. Sucrose phosphorylase from *Pseudomonas saccharophylia* was discovered[88] in 1943, causing a synthesis of sucrose from glucose 1-phosphate and fructose. A corresponding enzyme has never been found in plants and it was not until 1953 that sucrose synthesis from UDPG was demonstrated[36,89] in wheat seedlings (Figure 7).

Information on the biosynthesis of cotton cellulose has been obtained by the use of ^{14}C-glucose. Greathouse[90] introduced [1-^{14}C]glucose into *Gossypium herbaceum*. Forty-four per cent of the added [1-^{14}C]glucose was recovered in the cellulose and 99·97 per cent of the activity was associated with $C_{(1)}$ of the polymerized glucose, indicating a direct polymerization, that is, without preliminary breakdown of the carbon chain of glucose. Shafizadeh and Wolfrom[91] performed similar experiments, recovering up to 23·5 per cent of the radioactivity as cellulose, but they found that all the carbon atoms of the polymerized glucose had acquired some radioactivity, although the major amount was still associated with the original labelled carbon atom. When [1-^{14}C]glucose was the substrate the redistributed activity in $C_{(6)}$ (29 per cent) was roughly equal to the sum of activities in $C_{(2)}$–$C_{(5)}$. With [6-^{14}C]-glucose the recovered activities were approximately $C_{(6)}$ 69 per cent, $C_{(2)}$–$C_{(5)}$ 10 per cent, and $C_{(1)}$ 21 per cent. The tendency of the redistributed activity to become concentrated at the opposite end of the molecule was shown even more clearly in experiments with wheat seedlings.[92] [1-^{14}C]Glucose gave rise to cellulose in which the glucose units contained 70·9 per cent activity at $C_{(1)}$ and 22·3 per cent at $C_{(6)}$. The glucose units in cellulose synthesized from [6-^{14}C]glucose had 17·1 per cent of the labelling at $C_{(1)}$ and 72·2 per cent at $C_{(6)}$. This randomization of activity is thought to be due to glycolysis of part of the added glucose to triose phosphate, followed by resynthesis of hexose.

The mechanism of cellulose synthesis presents a problem not yet encountered in other types of polysaccharide synthesis, namely the insolubility of cellulose. Even a molecule as small as celloheptaose is not easily soluble in water.[93] It seems likely that the donor substrate for plant cellulose synthesis will be soluble and the kinetics of the reaction between a soluble (donor) and insoluble (acceptor) substrate, the growing cellulose chain, may prove to be abnormal, especially so since the only cellulose-synthesizing enzyme preparations known (from *A. xylinium*) are also insoluble.

DEGRADATION OF CELLULOSE

Just as our knowledge of the synthesis of cellulose lags behind that of starch, so is cellulose breakdown comparatively little understood. This is not for lack of interest in the subject. Cellulolysis has great practical significance. Cellulose is a main article of diet for many animals, who therefore are equipped with the means for its breakdown, and cellulolysis is also important in the microbial decay of cotton fabrics.[94–98] Pirie[99] has drawn attention to the commercial possibilities of a cellulase preparation in the utilization of the otherwise wasted cellulase of straw, grass and brushwood. In looking for sources of cellulose, attention is naturally directed to the digestive systems of animals and to microbial degradation of cellulose. Being a structural component in plants, it is not expected that the plant will metabolize cellulose to any great extent, although cellulolysis occurs during seed germination and plant cellulases have been prepared.[98] When herbivorous animals are examined for the source of cellulolytic activity, it is found that they rely for the breakdown on micro-organisms. This situation has been commented on by Pirie.[99]

> ‘The herbivorous vertebrates have an abundant potential energy source [cellulose] but they are unable to make direct use of it and have to adopt a variety of subterfuges before it becomes metabolically available to them. These encumber the animal with a great belly full of symbiotic micro-organisms whereas a cow or elephant that could secrete cellulase, with an activity comparable to that of the other enzymes it makes, along with these enzymes, from its pancreas or salivary glands, could be as slender as a greyhound and yet use cellulose as its primary energy source.’

The study of cellulases is therefore mainly concerned with those enzymes from micro-organisms. As to the reason why the animal itself does not secrete cellulase, Pirie[99] suggests that ‘ the integrity of the vertebrate gut may depend, in part, on a substance with a β-glucoside link in it, which would be destroyed if exposed to a soluble cellulase ’.

All known types of cellulase exert their action by hydrolysis. There is no evidence as yet for phosphorolytic breakdown.

The powerful cellulolytic action of the bacteria of sheep rumen can be reproduced in the laboratory with suspensions of the micro-organisms. Eighty per cent or more of added cellulose can be digested in relatively short periods by the intact or the non-viable organism.[100,101] So far, however, there seems to have been a complete failure to extract from the cells any enzyme preparation which can achieve either the same extent or the same rate of degradation of cellulose. Halliwell[100] noted only one example[102] in the literature prior to 1957 where more than 6 per cent hydrolysis of cellulose powder or cotton was achieved with a cell-free cellulase. This author[100] drew attention to the need to obtain a substantial hydrolysis of cellulose in order to be certain that the responsible enzyme is a cellulase. Simply to obtain a few per cent hydrolysis, measured by reducing power, is an unreliable guide because non-cellulosic impurities in the substrate, e.g. xylan,[103,104] may be undergoing hydrolysis, and not cellulose itself. Another difficulty associated with the use of insoluble cellulose as a test substrate is of obtaining a standard material for comparative purposes. For example, an extract from *Myrothecium verrucaria* caused 88 per cent solubilization of acid-swollen cellulose powder but when acting for a much longer time on the untreated powder caused only 11 per cent solubilization.[105] Similarly, *Stachybotrys atra* cellulase hydrolysed filter paper but not a highly crystalline cellulose (cotton duck).[106] Appreciable cellulolysis by cell-free extracts is in fact only observed with cellulose which has already been degraded, by acid (hydrocellulose), by ball-milling, or of cellulose rendered soluble by etherification. Carboxymethylcellulose is the most frequently used soluble ether. There is an obvious similarity of these findings to those with granular starch (see above), and as in the case of starch, it has been proposed that a special enzyme (C_1) attacks insoluble cellulose, rendering it susceptible to attack by a second type of cellulase, C_x.[94,95,107] The latter enzyme, it was postulated,[107] can attack a soluble derivative such as carboxymethylcellulose without assistance from the C_1 enzyme. Non-cellulolytic fungi were found to hydrolyse carboxymethylcellulose and these were thought to contain C_x but not C_1, cellulolytic fungi containing both enzymes. Preparations from cellulolytic fungi showed widely varying relative rates of attack on insoluble cellulose and carboxymethylcellulose.[94,95,107] This was ascribed to variation in the $C_1 : C_x$ ratio. Evidence against this hypothesis came from several sources. There was no change in the $C_1 : C_x$ ratio of a *M. verrucaria* cellulase preparation before and after heating at 100°.[108] It was argued that two enzymes (C_1 and C_x) would be unlikely to show an equal resistance to heat. Furthermore when *M. verrucaria* cellulase was purified to the extent that it was homogeneous in the ultracentrifuge and during electrophoresis, its relative activity towards soluble and insoluble celluloses remained the same as in the crude state.[109] The idea from these experiments that a single cellulase, and not two enzymes, is concerned with the hydrolysis of insoluble cellulose to sugars was reconciled with the results showing an apparently variable $C_1 : C_x$ ratio when it was found that the rate of attack of swollen cotton linters by *M. verrucaria* cellulase could be markedly influenced by the addition of enzymically inert proteins or by acidic or basic dyes.[110] These effects were much weaker when the substrate was a soluble (carboxymethylcellulose) substrate. The action of the proteins and dyes is thought to be due to their ability to alter the surface potential of insoluble cellulose and, in turn, the ability of the enzyme to attack the substrate.[110] In view of these findings the necessity for postulating two cellulose-degrading enzymes disappears. The multi-enzyme hypothesis has, however, recently been revived to explain the difference in optimum temperature of action of *M. verrucaria* cellulase when it acts on carboxymethylcellulose as opposed to cellulose powder.[101] It may be questioned, however, whether an enzyme will necessarily have the same temperature optimum with a soluble and an insoluble substrate. Another explanation of the differing properties of cellulase preparations derives from the many observations that single organisms contain two or more cellulases which can be separated by chromatography, electrophoresis, or other methods.[107,111–113] For example, *M. verrucaria* may contain three cellulases, separable by electrophoresis.[112] One of these corresponds to Whitaker's electrophoretically pure specimen.[109] All three have different $C_1 : C_x$ ratios.[112] In other words, this ratio may represent a characteristic property of a single enzyme, a property which is likely to vary with protein structure. Just as the α-amylases differ among themselves in their affinities for starch molecules, so may the individual cellulases differ in their relative affinities for soluble and insoluble cellulose.[112]

In view of the difficulties associated with the hydrolysis of insoluble cellulose it is not surprising that mechanistic studies of cellulase have been largely concerned with soluble substrates. Four types of enzyme acting on β-1 : 4-glucosidic linkages have been recognized. Three of these occur in *M. verrucaria*.

(1) The cellulase of *M. verrucaria* has little action on cotton linters.[109] It attacks acid-swollen linters (DP ~1,000)[114] and an insoluble degraded cellulose (DP 24)[115] more rapidly. Soluble cellodextrins and cellobiose are also hydrolysed.[116,117] These statements refer to the preparation found to be homogeneous in the ultracentrifuge and on electrophoresis (see above).[109] Other reports of the cellulase, however, say that it has no action on cellobiose. The

cellulase degrades by a random, as opposed to end-wise, action [109,114,116,119] (cf. α-amylase) and the end products are cellobiose and glucose, in equimolar proportion. (*S. atra* cellulase also acts at random.[106]) The cellulase does, however, show preferences for linkages in particular situations. For example, the non-reducing-end linkage of cellotriose is hydrolysed 5 times as rapidly as the reducing-end bond.[120] The sugars are released in the β-configuration.[121] An interesting property of the enzyme is that on being heated it loses activity towards cellodextrins in different degrees, the loss in activity being inversely related to the degree of polymerization of the substrate.[117] The relative rates of hydrolysis of cello-biose, -triose, -tetraose, and -pentaose by the unheated enzyme are 1 : 14 : 71 : 443. Under one set of heating conditions the activity towards cello-biose and -triose disappeared while that towards the -tetraose and -pentaose fell to 15 and 75 per cent, respectively, of the original values. Other conditions of heating caused still different relative effects. This finding obviously raises considerable complications in the study of the enzyme, since it may not be possible to obtain purified preparations with reproducible relative affinities for different substrates.

(2) Cellobiase activity is found in *M. verrucaria* culture filtrates and can be preferentially destroyed, preserving cellulase activity, by heat treatment.[108] This may be the activity of an enzyme different to the cellulase, or it may simply be that the heating impaired the action of the cellulase[117] (see paragraph (1)). The cellobiase activity is inhibited by high concentrations of cellobiose.[109]

(3) *M. verrucaria* culture filtrates form cellotriose from cellobiose.[108,118] (Other examples of transglycosylase action on cellobiose have been noted earlier.) It was not decided whether this action was due to an enzyme other than the cellulase or cellobiase[108] but other workers[118] have detected transferase activity in preparations devoid of cellobiase. Whitaker also found transferase activity in his electrophoretically pure *M. verrucaria* cellulase and made use of this to prepare ^{14}C-labelled cellotriose.[120] It is therefore uncertain whether the transferase activity is that of an enzyme distinct from cellulase.

(4) The fourth type of cellulase activity is shown by a crystalline enzyme from *Irpex lacteus*.[122] This has no cellobiase activity but has transferase activity. It splits *p*-nitrophenyl β-cellobioside into cellobiose, *p*-nitrophenol, glucose, and *p*-nitrophenyl β-glucoside and is said to hydrolyse cellulose by an end-wise action, liberating cellobiose and a little glucose. That it also attacks carboxymethyl cellulose is unexpected since β-amylase, which acts in an end-wise

manner on starch (see above), does not attack carboxymethyl starch.[123] Its action is impeded by the ether groups.

CONCLUSION

Starch

As we have seen, the main outlines of the biosynthesis of starch have now been defined. Some points of detail, such as the mode of synthesis of the α-1 : 3-linkages and the $C_{(6)}$-phosphate groups, remain to be explored. Attention must now be turned to the elucidation of the actual course of the *in vivo* biosynthesis, to investigate such questions as the precise function in Nature of the enzymes whose actions have been studied *in vitro*. For example, does phosphorylase play any part in starch synthesis? Is it instead an agent of degradation? Does the plant contain a UDPG-utilizing phosphorylase? Is the mechanism of starch synthesis the same for all plants? Why does the sweet potato contain β-amylase and the white potato not? Studies with the living organism are greatly assisted by tracer techniques and several such studies of the pathways between starch and sugars in living plants have been reported in recent years[28,30,37,124] and are likely to increase greatly in number. The temporary deposition of starch in the leaf, its translocation to a more permanent storage elsewhere in the plant are also aspects which lend themselves to tracer studies. Of particular interest is the structure of the starch granule,[125] the way in which it grows, and the variation in size between different plants. Tracer methods have shown that starch granules grow by apposition of layers to the outer surface rather than by intussusception (growing inwards).[126]

The degradation of starch *in vitro* is now well understood although very many details of the mechanism of degradation by the various hydrolases remain to be discovered. The work on the action patterns of the exo enzymes, e.g. β-amylase and phosphorylase, is likely to prove a model in studies of the end-wise splitting of other naturally occurring polymers such as proteins and nucleic acids. *In vivo* studies of degradation are likely to be concerned with the mode of dissolution of starch granules and the way in which plants prepare themselves to utilize their reserves of starch during germination, e.g. the rapid increase in the α-amylase content of barley during germination.[3]

Cellulose

The immediate problem in the study of cellulose biosynthesis is to discover the pathway in plants, and in animals also now that it has been discovered in bovine skin.[87] Problems of organization present themselves, as with starch granules. Why are there amorphous and crystalline regions in cellulose fibres? Do the amorphous regions represent points at which biosynthesis was so rapid that orderly deposition could not take place? What are the forces controlling the remarkable and complex pattern of orientation of cellulose fibres in *Valonia*?[127] In what way is cellulose biosynthesis bound up with that of the hemicelluloses? To what extent, if any, is there a turnover of cellulose in the plant?

In vitro experiments on the degradation of cellulose have so far failed to reproduce the rapidity with which intact micro-organisms can assimilate the polysaccharide. The aim must be to imitate the *in vivo* process and purification and crystallization of more of the responsible enzymes would be a profitable first step. It would then be much easier to investigate the special problems involved in the degradation of an insoluble substrate, and the question of the multi-enzyme nature or otherwise of the degradation of cellulose might thereby be solved.

Problems of enzyme action pattern are also much easier to solve with pure enzymes. It would seem profitable to devote particular attention to the cellulases of rumen micro-organisms, none of which has yet been obtained in anything but an apparently very low state of purity.

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